Diploma thesis

Congenital adrenal hyperplasia

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Diagnosis, treatment, course of disease in Styrian patients born between 1992 and 2011

submitted by

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ABSTRACT

Introduction:

Congenital adrenal hyperplasia (CAH) is an autosomal-recessive enzyme disorder of cortisol synthesis within the adrenal gland. The disorder results in elevated androgen levels and can also include decreased aldosterone levels. 21-hydroxylase deficiency is responsible for more than 90% of cases. There is a classic salt-wasting form and a classic simple-virilising form, as well as a non-classic late-onset form and an asymptomatic form. The incidence of classic CAH is approximately 1:12.000 and carrier frequency is 1:60 in Caucasians. Symptoms include ambiguous external genitalia and virilisation in girls, as well as precocious puberty, short stature and infertility in both sexes. Salt-wasting may be a life-threatening condition in infants. Since 2001, CAH is included in the Austrian newborn screening based on the measurement of 17α-hydroxyprogesterone.

Materials & methods:

We collected data of 18 affected children born between 1992 and 2011 in Styria. We put our emphasis on weight, height and bone age, as well as time of and reason for diagnosis. Moreover, we compared the prevalence of CAH before and after introduction of screening.

Results:

There were twelve girls and six boys diagnosed with CAH in Styria from 1992 to 2011.
In group A (patients not screened), CAH was diagnosed in six children (m 3; f 3); four with salt wasting (SW) 21-OH deficiency (m 2; f 2) and two with simple virilising (SV) 21-OH deficiency (m 1; f 1). Calculated prevalence of CAH was 1:19.833 in this group.
In group B, CAH was diagnosed in twelve children (m 3; f 9). Eight of them were diagnosed with SW 21-OH deficiency (m 2; f 6), two with SV 21-OH deficiency (m 0; f 2) and two with 11β-hydroxylase deficiency (m 1; f 1). Prevalence of 21-hydroxylase deficiency in total was 1:10.322, prevalence of SW 21-hydroxylase deficiency was 1:12.903, and prevalence of 11β-hydroxylase deficiency was 1:51.614. The gender ratio in group A was 1:1, whereas it was 2:1 in group B.
Diagnosis in girls was made due to ambiguous external genital in the first instance and due to growth abnormalities and precocious puberty in boys before screening. Since screening has been introduced, boys have been diagnosed and treated earlier.
To evaluate height and weight, triennial data were collected. In not-screened boys, height SDS was -0,77 ± 4,14 and weight SDS -1,34 ± 2,82. In screened boys, it was -1,32 ± 5,64 respectively -1,57 ± 3,22. In not-screened girls, height SDS was -0,57 ± 2,45 and weight SDS +0,08 ± 1,87. In screened girls, it was -2,22 ± 1,21 respectively -2,75 ± 1,85.

Bone age was accelerated in six out of eleven tested female patients and in four out of five tested male patients. Deviation of bone age from chronologic age was higher in boys, with levels between +3,25 years and +6,36 years on average, whereas it was +0,2 to +3,7 years in girls on average.

Discussion & conclusion:

Newborn screening for CAH is efficient for diagnosis and increases the rate of detection of CAH. Screening apparently has greatest benefit for boys.

Height and weight outcome as well as bone age are still not optimal in congenital adrenal hyperplasia, even though adequate and consequent therapy seems to improve the outcome.

ZUSAMMENFASSUNG

Einleitung:

Das adrenogenitale Syndrom (AGS) ist ein autosomal rezessiv vererbter Enzymdefekt der Cortisolsynthese, welcher mit erhöhtem Androgenspiegel sowie in manchen Fällen vermindertem Aldosteronspiegel einhergeht und die Nebenniere betrifft. Die 21-Hydroxylase ist für über 90% der Fälle verantwortlich. Es wird unterschieden zwischen einer klassischen Form mit Salzverlust, einer klassischen Form mit Virilisierung, sowie einer nicht klassischen late-onset Form, und einer asymptomatischen Form. Die Inzidenz der klassischen Form unter Kaukasiern liegt in etwa bei 1:12,000, die Trägerfrequenz bei 1:60. Zu den Symptomen zählen vergrößerte äußere Genitalien und Virilisierungszeichen bei Mädchen, sowie Pubertas praecox, reduziertes Längenwachstum und Fertilitätsprobleme bei beiden Geschlechtern. Außerdem stellt der Salzverlust eine potentiell lebensbedrohliche Komplikation insbesondere für Säuglinge dar. Die Erkrankung wird seit 2001 im österreichischen Neugeborenen-Screening untersucht, basierend auf der Messung von 17α-Hydroxyprogesteron-Spiegeln.

Material & Methode:

Daten von insgesamt achtzehn PatientInnen wurden gesammelt, welche zwischen 1992 und 2011 in der Steiermark geboren wurden. Das Hauptaugenmerk zur Beurteilung der Erkrankung wurde auf Größe, Gewicht und Knochenalter, sowie den Diagnosezeitpunkt und die Ursache für die Diagnosestellung gelegt. Außerdem wurde die Prävalenz des AGS vor und nach Einführung des Screenings erhoben.

Resultate:

Bei zwölf Mädchen und sechs Jungen in der Steiermark, welche zwischen 1992 und 2011 geboren wurden, wurde ein AGS diagnostiziert. In Gruppe A (nicht gescreent), wurde die Erkrankung bei sechs Kindern festgestellt (m:3, f:3), wovon vier (m:2; f:2) an der SW Form (mit Salzverlust) und zwei (m:1; f:1) an der SV Form (ohne Salzverlust, mit Virilisierung) des 21-Hydroxylase-Mangels litten. Die berechnete Prävalenz in dieser Gruppe betrug 1:19.833. In Gruppe B (gescreent) wurde das adrenogenitale Syndrom bei zwölf Kindern festgestellt (m 3; f 9). Acht von ihnen litten an der SW Form (m 2; f 6), zwei an der SV Form des 21-Hydroxylase-Mangels (m 0; f 2) und zwei an 11β-Hydroxylase-Mangel (m 1; f 1). Die berechnete Prävalenz des AGS aufgrund 21-Hydroxylase Mangels gesamt liegt bei 1:10.322, die Prävalenz der SW Form bei 1: 12.903. Die Prävalenz des 11β-Hydroxylase-Mangels liegt bei 1:51.614. Das Geschlechterverhältnis in Gruppe A ist 1:1, wohingegen es in Gruppe B bei 2:1 liegt.

Vor Einführung des Screenings erfolgte die Diagnosestellung bei Mädchen vor allem durch nicht eindeutigen bzw. vermännlichten Genitalbefund, bei Jungen durch Pubertas praecox und abnormale Wachstumsschübe. Seit der Einführung des Neugeborenen-Screenings erfolgen Diagnose und Behandlung vor allem bei betroffenen Jungen früher.
Zur Evaluierung von Größe und Gewicht wurde Daten im Abstand von drei Jahren beurteilt. Bei den nicht gescreenten Jungen lag der Standard Deviation Score (SDS) der Größe bei -0,77 ± 4,14, und der SDS des Gewichts bei -1,34 ± 2,82. Bei den gescreenten Jungen lag der SDS bei -1,32 ± 5,64 bzw. -1,57 ± 3,22. Bei den nicht gescreenten Mädchen lag der SDS der Größe bei -0,57 ± 2,45 und der SDS des Gewichts bei +0,08 ± 1,87. Bei den gescreenten Mädchen lag der SDS bei -2,22 ± 1,21 bzw. -2,75 ± 1,85.
Das Knochenalter war bei sechs von elf Mädchen und bei vier von fünf Jungen akzeleriert. Die Abweichung des Knochenalters vom chronologischen Alter war bei Jungen höher als bei Mädchen, mit durchschnittlichen Werten zwischen +3,25 Jahren und +6,36 Jahren bei Jungen und +0,2 und +3,7 Jahren bei Mädchen.

Diskussion & Zusammenfassung:

Das Neugeborenen-Screening ist effizient für die Diagnose des AGS ist und die Rate der Diagnosestellungen wird dadurch erhöht. Jungen profitieren am meisten von den Screeningmaßnahmen.
Das Outcome bezüglich Größe, Gewicht und Knochenalter bei PatientInnen mit AGS ist trotz adäquater und konsequenter Therapie noch immer nicht optimal, scheint sich jedoch zu verbessern.

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Abbreviations

17-OHP	17-hydroxyprogesterone
ACTH	adrenocorticotropic hormone
AGS	adrenogenitales Syndrom
BMI	body mass index
CAH	congenital adrenal hyperplasia
CRH	corticotropin releasing hormone
CRF	corticotropin releasing factor
CVS	chorionic villus sampling
CYP	cytochrome P
DHEAS	dehydroepiandrostenedione
DNA	deoxiribo nucleic acid
DOC	deoxycorticosterone
HC	hydrocortisone
HPA	hypothalamic-pituitary axis
HSD	hydroxy-steroid-dehydrogenase
MR	magnetic resonance
OH	hydroxy /hydroxylase
PCO	polycystic ovaries
SDS	standard deviation score
SV	simple-virilising
SW	salt-wasting
TART	testicular adrenal rest tumour

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1 Congenital adrenal hyperplasia – introduction

Congenital adrenal hyperplasia is a hormonal disorder of the adrenal gland. It characterizes enzyme deficiencies affecting the sex steroid, glucocorticoid and possibly mineralocorticoid formation in the adrenal cortex. (1,2)

There are several disorders of hormone production affecting the adrenal gland, however the most common ones are the ones affecting the corticosteroid production. These disorders are shortly described as the CAHs (congenital adrenal hyperplasia). (1)

The adrenal gland consists of cortex and medulla, both synthesizing respectively excreting various hormones. The medulla produces catecholamines and the cortex steroids. The latter consists of three different parts which produce different hormones: the zona glomerulosa, being the external unit, synthesizes mineralocorticoids such as aldosterone, the zona fasciculata in the middle produces cortisol, and the zona reticularis on the inside generates various sex steroids. In congenital adrenal hyperplasia, hormone production is modified at a various extent. (1,3)

Via the hypothalamic-pituitary-adrenal axis, cortisol levels are regulated via a feedback mechanism. Blood cortisol level is the determining factor, regulating the secretion of corticotropin-releasing hormone (CRH) from the hypothalamus and adrenocorticotropic hormone (ACTH) from the pituitary. A lack of cortisol leads to feedback and increased excretion of CRH and ACTH, furthermore the adrenal tissue is stimulated in order to raise cortisol production. In CAH, this mechanism is disturbed. (3)

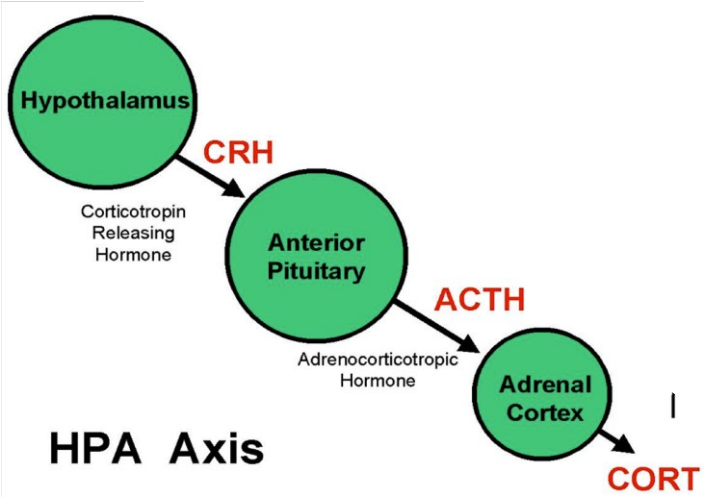

Figure 1: HPA axis – regulation of hormone synthesis, modified. (4)

CAH is caused by autosomal recessive heredity transmission. One of the enzymes included in the cortisol synthesis is affected by various mutations. The chromosomes carrying the inducing mutations have been identified. (1,5,6)

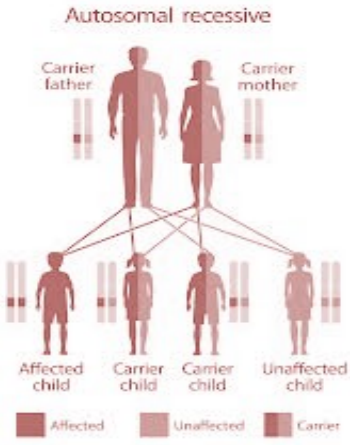

Figure 2: Autosomal recessive inheritance (7), modified.

If parents are carriers, 25% of children will be affected and 50% will be carriers themselves, following the Mendelian laws. (8)

2 Pathophysiology

In CAH, the secretion of cortisol is reduced, which leads to increased ACTH levels. This hormone is generated from the fore lobe of the pituitary. In order to stimulate the adrenal gland to produce sufficient amounts of cortisol, ACTH levels rise. As a consequence the adrenal tissue is enhanced due to intensified stimulation. (6,9)
However these mechanisms still cannot compensate the lack of cortisol by stimulating the adrenal cortex and also not generate sufficient pulsatile cortisol production. The hormonal imbalance can lead to various symptoms, starting from ambiguous genitalia both in male and female infants, as well as early puberty and impaired reproduction due to sex steroid excess, and possibly causing problems in homoeostasis and blood pressure due to mineralocorticoid disorder. Therefore, hormonal substitution is needed in affected patients, which may cause further medical problems, e.g. metabolic consequences, growth problems, reproduction problems etc. due to high glucocorticoid doses. (1,2)
Generally, one can say that the pathophysiology depends on the severity of enzyme loss. Cholesterol is the basic module of all descending steroid products. Most of the enzymes converting the different substrates into each other in the cortisol pathway are cytochrome P450 dependent. The genes encoding the different enzymes lie on various chromosomes. (10)

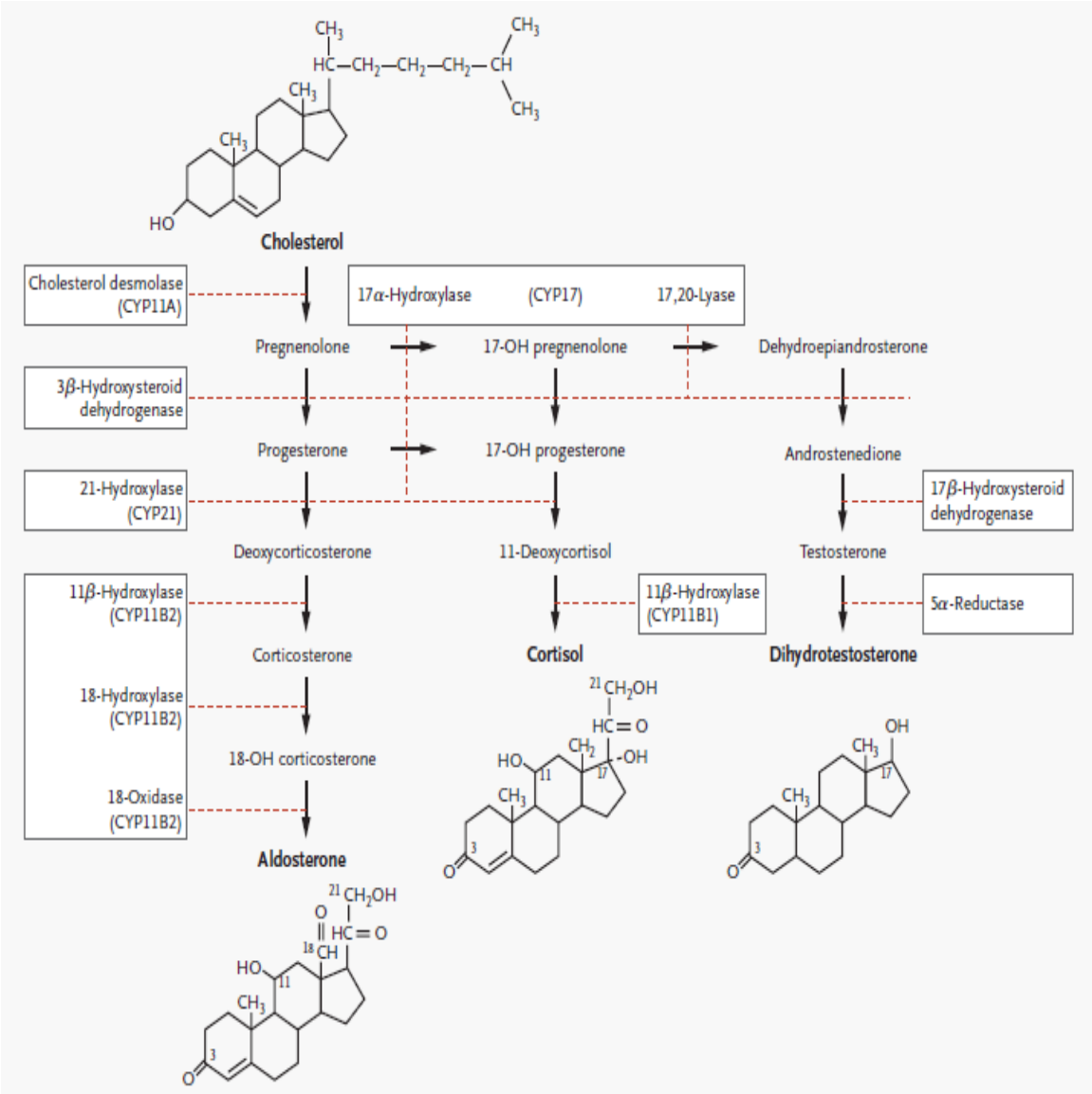

Figure 3: Pathways of steroid biosynthesis (5)

3 Epidemiology

The enzyme disorders in the cortisol pathway causing CAH refer to defects of the 21-hydroxylase in 90-95%. (2,3,10)

In 3-5% of CAH cases, there is a defect of the 11β-hydroxylase, being the second most frequent enzyme affected besides 21-hydroxylase. (10)

There are also defects in 3β-hydroxylase, 5α-reductase, 17α-hydroxylase and 17,20-lyase causing CAH, as well as a form called lipoid CAH. These occur rather to very rare. (1)

Approximately one out of 15,000 newborn infants worldwide suffers from the severe CAH due to 21-hydroxylase deficiency, as the results of worldwide screening in about 6.5 million babies have shown in thirteen countries all over the world (e.g. France, Japan, New Zealand etc.). Approximately one out of sixty individuals carries the affected gene, meaning that he or she has heterozygous alleles for the autosomal-recessive disorder. (11)

The salt-wasting type is estimated to be the most common one, affecting 75% of patients with disorders of 21-hydroxylase. (5)

In a study by Pang et al., it has been found that salt-wasting occurs in 67% and non-salt-wasting in 33%. (11)

Mild, non-classic form is only detected insufficiently in neonatal screening, although it is assumed to occur much more often than the severe form. In a study by Speiser et al., it was found that approximately 0.1 per cent of Caucasian people are affected. (12)

However 21-hydroxylase deficiency ranks among the most frequent autosomal-recessive affections. (1)

11β-hydroxylase disorder is as frequent as one in 160,000 newborn infants. Frequency of other forms cannot be estimated because data on the issue are lacking. Concerning the sex of affected patients, the gender ratio is balanced with female and male children being affected at the same extent. (10)

CAH form	Frequency
21-hydroxylase deficiency	1:14,199
11β-hydroxylase deficiency	1:160,000
3β-hydroxysteroid deficiency	uncertain
5α-reductase deficiency	uncertain
17α-hydroxylase deficiency	uncertain
17,20-lyase deficiency	uncertain
Lipoid CAH	uncertain

Table 1: Frequency of CAH forms (10,13), modified.

As a result of newborn screening for CAH in several populations all around the world, “classic” CAH was found to occur at a frequency of 1:14,199 live births. (13)

4 Forms of CAH

In general, cortisol and sex steroid secretion is always affected in CAH at a various extent. Moreover different CAH forms may show distinctive signs and symptoms.
Most common enzyme deficiencies affecting the cortisol pathway are 21-hydroxylase deficiency, 11β-hydroxylase deficiency and 17α-hydroxylase deficiency, which is rather rare. What they have in common is elevated production and secretion of androgens in the adrenal gland due to enzyme deficiency with partly or total loss of cortisol production. (14)

4.1 21-hydoxylase-deficiency

CAH caused by 21-hydroxylase deficiency is the most common form of CAH (95% of patients affected). Basically, the pathologic alterations include elevated sex steroids, decreased glucocorticoids, and possibly decreased mineralocorticoids, referring to whether or not aldosterone production and secretion is impaired. There are three different forms of 21-hydroxylase deficiency: the classic salt-wasting form, the classic simple-virilising form, and the non-classic form including late-onset-CAH as well as asymptomatic forms without clinical relevance for the individual. (2,3)
There is no positive discrimination to a certain sex, males and females are affected at the same extent and gender ratio is 1:1. (3)
Also, there is a distinction between classic and non-classic CAH: the former already shows symptoms as soon as birth, the latter occurs later on and at a minor extent. (1)

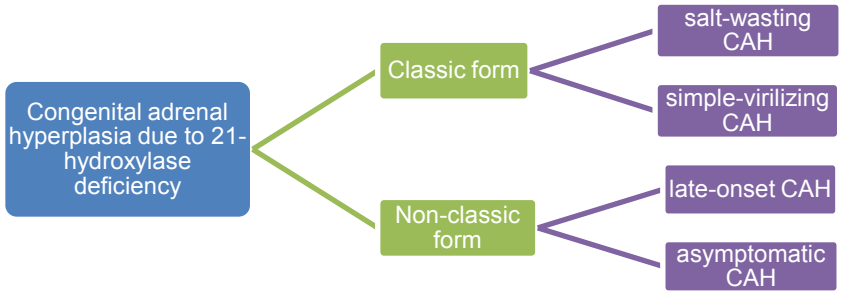

Figure 4: Forms of CAH due to 21-hydroxylase deficiency (3)

4.1.1 Genetics

The assignable genetic cause for 21-hydroxylase deficiency is located on the short arm of chromosome 6. (8)

Besides the active gene locus CYP21A2, there is also an inactive pseudo-gene CYP21A inactivated due to mutation. These two are rather similar in their genetic configuration. It is suggested that gene parts are exchanged between the two genes and that loss of function may be the result of such gene conversions. (10)

So far, 94 different mutations have been described to cause 21-hydroxylase deficiency, whereas only ten of these mutations are responsible for about 95 per cent of all cases of classic 21-hydroxylase CAH. (15)

4.1.2 Pathophysiology

Basically, 17-hydroxyprogesterone is not turned into 11-deoxycortisol. Thus there is an accumulation of cortisol pre-stages such as pregnenolone, progesterone, 17-hydroxypregnenolone and 17-hydroxyprogesterone due to sustained stimulation of the adrenal gland by ACTH. This is a result of the feedback-mechanism caused by low cortisol levels. The pre-stages are turned into androgens instead. (1)

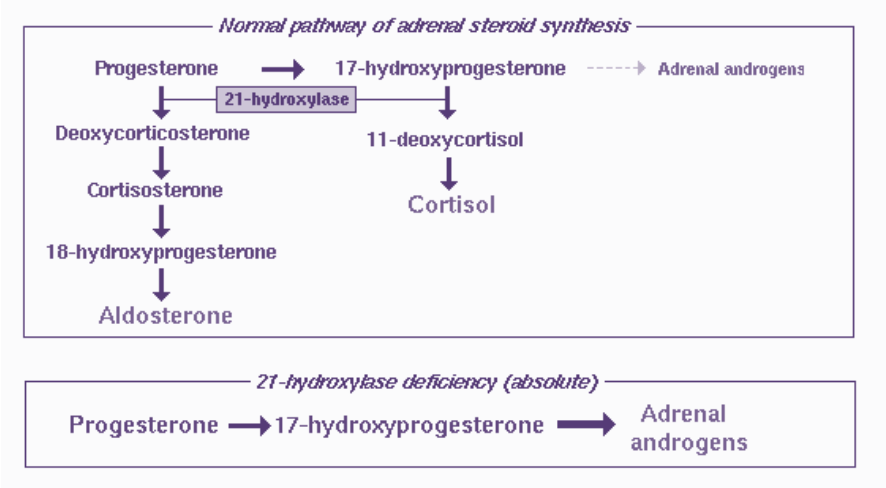

Figure 5: Effect of 21-hydroxylase on pre-stage accumulation (16), modified.

4.2 Classic salt-wasting form

In classic CAH with salt wasting, there are elevated androgen levels, a lack of cortisol, and decreased mineralocorticoid levels. Precisely, there is a compilation of low sodium levels, high potassium levels, lack of aldosterone and compensative high renin activity. In addition, there can also be lack of catecholamines, for their formation is stimulated by cortisol. (5)

4.2.1 Clinical features

Basically the peculiarity depends on the dimension of the enzyme dysfunction respectively the kind of underlying mutation, differing concerning enzyme rest activity. (8)
The classic form shows symptoms in affected children early due to accumulation of glucocorticoid pre-stages and sex steroids, as well as electrolyte imbalances. The mild form may only occur later in childhood. (3,17,18)

Females

Female newborn babies manifest enlarged external genitals at a various extent as a result of sex steroid excess during pregnancy. CAH is the most common reason for such findings in female infants. (2)

Males

Male newborn babies usually do not show symptoms when born, but may also be found with penile enlargement or a hyperpigmentated scrotum. (5)
Therefore, the detection of CAH in boys depends on the loss of mineralocorticoids and salt-wasting, which may lead to a metabolic crisis usually in the first two weeks of life. (2)

4.2.2 Diagnosis

Diagnosis due to clinical features happens earlier in girls because of their ambiguous genitalia. Boys are usually diagnosed later, eventually due to an adrenal crisis caused by lack of mineralocorticoids and cortisol, or show early pubertal development, possibly at around three years of age, if not affected by the salt-wasting form. (2,3)
Moreover, growth is also impaired in these patients, even if therapy is adequate and supervised. There is a premature closure of epiphyseal cartilage in undertreated CAH due to androgen excess and growth inhibition in overtreated CAH due to cortisol excess, both resulting in decreased height outcome. Patients often do not accomplish their target height, in many cases not being as tall as their parents. (5)

4.2.3 Salt-wasting crisis

The condition of salt-wasting can also lead to a so-called salt-wasting crisis, meaning a condition of low glucose levels and low fluid volume due to shortage of glucocorticoids and mineralocorticoids. It can affect both girls and boys, although being more likely to affect males as they do not present with abnormally formed genitals at birth. Thus they may not be diagnosed with CAH as soon as girls and a salt-wasting crisis may be the first symptom of the underlying disease. (1)

Typical symptoms of salt-wasting are nausea and vomiting, loss of appetite and decreased weight, low sodium and high potassium levels, and the affected babies may also be dehydrated. The newborn infant shows symptoms within the first days, respectively weeks, of life. (2,3)

Therefore it is advised to keep newborn babies in the hospital for roughly one week until their electrolyte levels are balanced. Salt-wasting crisis can occur in older children due to physical or psychic stress, illness, injuries, inoculation, surgical procedures, and certainly by non-compliance in therapy or deficient therapy. (1)

4.2.4 Hypoglycaemia

This condition mostly occurs in newborn babies that suffer from CAH. As glucocorticoids have an effect on catecholamine excretion, lack of glucocorticoids leads to lack of catecholamines and consecutively hypoglycaemia. (2)

Even with adequate therapy and parental care, approximately 8 to 9 % of CAH patients show hypoglycaemia in early childhood. Also, stress situations, e.g. illness or exercise, as well as lack of hormonal substitution triggers low blood glucose levels. (2,19)

4.3 Classic simple-virilising form

In classic CAH without salt wasting, only cortisol synthesis is impaired, whereas mineralocorticoid synthesis appears adequately. The extent of virilisation due to androgen excess varies. The genital alterations in girls vary from a hypertrophic clitoris to a fully developed penile formation, whilst genotype and inner organs are clearly female. (3)

4.3.1 Clinical features

In SV-CAH, virilisation, augmented bone maturity and fast growth spurt are remarkable features. (18)

Females

In girls, there are enlarged external genitalia at birth. Hence female patients are diagnosed early. Signs of androgen excess can occur in addition. (18)

Males

In boys, there are no pathologic genital findings at birth, therefore they are diagnosed later due to early onset of puberty. (18,20)

Moreover, penile enlargement in boys can occur later on in childhood. (3)

4.4 Non-classic late-onset form

This form is referred to as late-onset CAH showing no genital abnormalities in newborn infants, i.e. newborn girls. Moreover it is considered to be less serious than the both forms mentioned above. (1)

Speiser et al. found that non-classic 21-hydroxylase deficiency appears more often than the classic form, approximately affecting one in 8,000 births. (12)

Non-classic CAH is likely to show shortly before puberty, although it may occur any time during childhood. However, sex steroid level is lower and clinical features are expressed to a lesser degree than in the classic form. (1,18)

4.4.1 Clinical features

The signs and symptoms are various and may begin at any time in any magnitude. In most cases patients seek medical advice just before the time of puberty. (6)
The non-classic form of CAH shows symptoms only in older children as late as in adolescence. As there is no lack of cortisol due to sufficient compensation respectively milder enzyme deficiency, androgens are mainly responsible for symptoms like pubertas praecox, altered bone maturity, acne, early pubic hair growth, baldness and impaired reproduction in both sexes, moreover hirsutism, polycystic ovary syndrome, period irregularities such as amenorrhea or oligomenorrhea in women and suspicious testicular adrenal tissue formations in men. (17)
Another typical feature is fast growth in childhood and early stop of growth in adolescence or adulthood. Therefore the patients are tall as a child and rather small as an adult. (3,17)

Females

Symptoms in girls range from hirsutism, alopecia, infertility, dysmenorrhea to polycystic ovary syndrome. (6)
Moreover, non-classic CAH is very difficult do distinguish from polycystic ovary syndrome. There is no reliable differentiation according to clinical features and further investigations are necessary. (21)

Males

Hypoplastic gonads and impaired fertility may occur in boys. (3)
Furthermore boys present with extended body height, early growth of pubic hair and beard as well as early puberty. Men are more difficult to diagnose, being most likely to present with infertility and shortness. (1)
In general, one can say that fertility in individuals affected by non-classic CAH can be reduced at a various extent both in males and females. In women, dysmenorrhea occurs frequently, whereas men show impaired sperm quality. (18)

4.5 Virilisation

As the developing foetus, more precisely the outer genitals, are sensible to androgens, there is already a remarkable enlargement detectable in females in utero. The alterations are described in the Prader characterization, varying from an ambiguous clitoris to a shaped phallus enclosing a urethra. (1)

Prader I	Prader II	Prader III	Prader IV	Prader V
Clitoris hypertrophy	Partial fusion of labia majora	Urogenital sinus at posterior end of small vulva	Small urogenital sinus at base of enlarged phallus	Penile urethra Phallic clitoris with glans

Table 2: Prader stages (1,22), modified.

Classification of virilisation in female CAH patients.

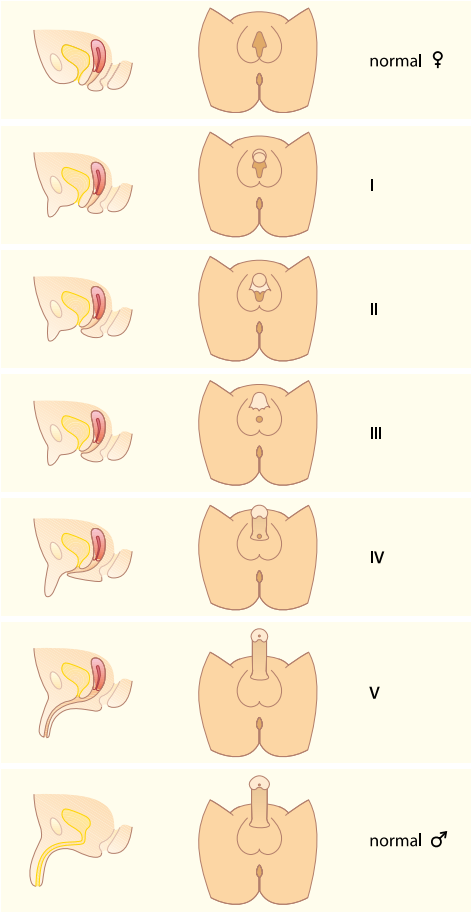

Figure 7: Prader classification in CAH patients (23)

Male genitals seem unaffected at birth, whereas increased androgen levels lead to penile enlargement and underdeveloped testes later on.
In both sexes, ACTH can lead to genital skin alterations i.e. amplified pigmentation.
Albeit the outer genitals are affected, the inner genitals are basically normally developed. (1)

4.6 Surgical intervention

Children born with any genital abnormalities, including ambiguity or enlargement, should be checked for the medical origin of the condition as soon as possible. This is essential not only for the possibility of early diagnosis and adequate medical treatment but also for the parents. An uncertainty of sex and an unknown medical condition are very distressing, therefore there has to be immediate clarification, respectively karyotyping giving information on sex. (1)

4.6.1 Surgery in females - necessity

Surgical procedures are recommended to be considered well before being planned and performed. It is important to include parents in decision finding. Surgical procedures should target sex-related appearance of external genitalia, adequate urinary tract function, and unaffected ability for intercourse, fertility and childbearing. Moreover, blood should be able to flow during menorrhea. Therefore procedures, e.g. vaginal dilatation, must be completed before puberty. It is important to guarantee normally shaped and well-functioning genitalia, but surgery is not necessary in every case, especially not in female infants with a low degree of virilisation, for enlargement may regress under adapted medical treatment.
However surgery should be a one-time event including all necessary corrections, e.g. correction of the clitoris or labiae. In addition is important to state that surgery should only be performed in experienced institutions (1,24)

4.6.2 Time of intervention

Surgical therapy is seen controversial and should be performed early in affected females with enlarged genitalia. The procedures should be initiated from the age of eight weeks until half a year. Within this time period, correction is less complicated. From the age of one year until adolescence surgical procedures are to be omitted unless there are health problems resulting from the condition. (24)
Surgery depends on shape of external genitals, ranging from correction of a hypertrophic clitoris to vaginal amelioration. (3)
However if there is no early diagnosis and children grow up with ambiguous external genitals, surgical intervention is not recommended above the age of two years. (1)

4.7 11-hydroxylase-deficiency

Cortisol and aldosterone synthesis is impaired, i.e. blocked, and pre-stages begin to rise. Elevated levels of 17-OH-progesterone can be found as well as 11-deoxycortisol and 11-deoxycorticosterone, and androgen levels comport themselves like in 21-hydroxylase-deficiency, presenting the same symptoms. (3)
11β-hydroxylase deficiency is the second most common form of CAH. (25)

4.7.1 Clinical features

This disorder may not cause salt-wasting for there is DOC compensating the lack of aldosterone and thus providing appropriate mineralocorticoid effect.
Elevated blood pressure profiles are the most important symptom, accompanied by a weak plasma renin activity and low potassium levels. Early puberty onset, rapid growth spurts and advanced bone maturity may occur in both sexes. In girls, early virilisation is common and in boys, gynaecomastia can be observed. (10,25)
Height in adulthood is severely impaired both in males and females. (26)
Fertility is not impaired, and quality of life is just normal, retaining life-long therapy. (3)
However children with high blood pressure profiles and augmented adrenal tissue should be sent to screening for 11β-hydroxylase deficiency. (25)

4.7.2 Genetics

The CYP11B1 gene causing 11β-hydroxylase deficiency lies on the long arm of chromosome 8. About thirty different mutations within this gene have been identified to cause this form of CAH. (10)

4.8 17-hydroxylase/17,20-lyase deficiency

This enzyme deficiency leads to a combination of decreased levels of androgens, oestrogens and cortisol. (27)
It exists either as deficiency in combination of the two enzymes mentioned above or isolated. Dysfunction of 17-α-hydroxylase leads to accumulation of pregnenolone and progesterone, initializing high levels of mineralocorticoids and leading to high blood pressure and volume expansion. Dysfunction of both 17-α-hydroxylase and 17,20-lyase may cause the same. Isolated dysfunction of 17,20-lyase shows normal levels of cortisol and aldosterone, yet androgen levels are depressed. Over all, there is a metabolic alkalosis. (1,3)
Currently, about 130 cases of this CAH from have been reported worldwide. (10)

4.8.1 Clinical features

Loss of androgens causes hypoplastic genitals respectively pseudohermaphroditism in boys and impaired sexual development in girls for both oestrogens and androgens are lacking. Moreover, puberty is delayed. (1,3,10)
Potassium levels are too low and blood pressure is often too high, in many cases being the first symptom detected. (27)

4.8.2 Genetics

Both enzymes are encoded by the same gene located on chromosome ten called CYP17. Around 50 different genetic alterations within the gene have been detected. (10)

4.9 3β-hydroxysteroid-dehydrogenase-deficiency

Dysfunction of 3β-hydroxysteroid dehydrogenase leads to accumulation of precursors like pregnenolone, 17-hydroxypregnenolone and DHEAS. They are not transformed into progesterone, 17-hydroxy-progesterone and androstenedione in a sufficient way. As a result, there is a lack of cortisol and aldosterone, possibly leading to salt-wasting. (1,27)

4.9.1 Clinical features

In boys, pseudohermaphroditism respectively depauperated genitals can be found due to lack of androgens. Moreover, hypospadia may occur due to lack of testosterone.
In girls, there is a mild hypertrophy of the clitoris, and symptoms of hyperandrogenaemia often showing as late as adulthood, e.g. hirsutism or amenorrhea. Female external genitalia may also be unaffected or only slightly virilised. However they are affected by conversion of high amounts of precursors into potent steroids in the periphery respectively high DHEAS levels. (1,3,10)

4.9.2 Genetics

Two genes named 3β-HSD-enzyme 1 and 2, lying on the short arm of chromosome 1, have been identified to cause 3β-HSD deficiency. There are around 34 mutations known to cause it. (10)

4.10 Lipoid CAH

Lipoid CAH is a form of steroid deficiency, where there is no adequate formation of any subsequent steroids in the steroid pathway beginning from cholesterol. There is a lack of androgens, mineralocorticoids and glucocorticoids, resulting in a female appearance of affected individuals, independently from actual sex of the individual. (28)
This form of CAH appears very infrequent. However, there are a larger number of cases reported in Korea, Japan and Palestine. (29)

4.10.1 Clinical features

Infants show symptoms of absolute cortisol deficiency and severe salt wasting. Girls may have unaffected genitalia, whereas boys show an intersexual phenotype. There is often a yellow discoloration of the adrenal gland due to accumulating cholesterol in tremendous amounts. (10)

4.10.2 Genetics

The affected gene is called CYP11A on the short arm of chromosome 8. The affected gene is the so-called StAR gene. StAR stands for steroidogenic acute regulatory protein. (10,30)

4.11 Non-classic CAH forms

Non-classic CAH or late-onset CAH is possible in 21-hydroxylase-, 3β-HSD- as well as in 11β-hydroxylase disorders. Symptoms are mostly similar to the classic form, whereas there are unaffected, unambiguous external genitalia in newborn infants. More severe symptoms may show as late as in puberty or at expected time of puberty. In women, androgen excess may lead to fertility issues, dysmenorrhea and hirsutism. (10)

5 Therapy

As the providing of lacking hormones is of utmost importance, glucocorticoid therapy in CAH patients is the centre of medical treatment. The effect is the replacement of cortisol and the suppression of ACTH. (31)
Salt-wasting forms need mineralocorticoid administration in addition and salt replacement as babies and children. (32)

5.1 Glucocorticoid therapy

Glucocorticoids have to be substituted life-long in congenital adrenal hyperplasia. (3)
They are administered to replace missing substrates and to inhibit a rush of androgens. The therapeutic effect is an improvement of final height outcome, an unimpaired reproduction and a reduction of genital ambiguity respectively increase of size in girls. (24)
Glucocorticoid dosing however should be moderate, otherwise treatment may result in Cushing's syndrome and small stature as well as pubertas tarda. (31)
There are different forms of glucocorticoid substitution. Hydrocortisone is given to children, whereas prednisone and dexamethasone can be given as an alternative when the patients are older. (33)
In small children, hydrocortisone is used. Up to 25 mg per m^2 and day can be necessary for an adequate prevention of androgen excess, however dosing between 10 and 15 mg per m^2 and day administered in three portions a day is most common in CAH. (5,24)
In older children and adolescence, long lasting glucocorticoid preparations are preferred for treatment of CAH. These long lasting substances, e.g. prednisone and prednisolone, should be administered two times a day. Dosing is between two and four mg per m^2 and day. Dexamethasone dosing is between 0.25 and 0.325 mg per m^2 and day and administered one time per day. (24)
In 21-hydroxylase, 11β-hydroxylase and 3β-HSD disorders, the glucocorticoid substitution is needed to prevent elevated androgen levels and thus augmented bone maturity, early growth ending and symptoms of masculinization in girls. Moreover, achieving a normal body shape and going through normal pubertal development can be enabled by glucocorticoid therapy. In 17α and 11β disorders showing high blood pressure, adequate medical treatment with glucocorticoids can lead to a remission of hypertension. (1)

5.2 Mineralocorticoid therapy

Fludrocortisone respectively Astonin H ® or Fludrocortison ® is administered to treat lack of mineralocorticoids. The dose reaches from 50 to 200 µg a day, dependent on age (infants 0,05 – 1; children around 0,1; adolescents/adults 0,1 – 0,15). In children, higher doses of fludrocortisone are needed than in adolescence or adulthood, however doses are not adjusted to size. (2,10,24)

Mineralocorticoids are given to all newborn infants suffering from the classic form of 21-hydroxylase deficiency. Fludrocortisone therapy should be started as soon as diagnosed with CAH. As a result, antidiuretic hormone and ACTH will show lower levels. Moreover, the glucocorticoid dose can be reduced when administering an adequate mineralocorticoid dose. Therefore Fludrocortisone is also used in non salt-wasters to enable smaller doses of glucocorticoids, because mineralocorticoids augment the effect of glucocorticoids. The maintenance of mineralocorticoid therapy is to be re-evaluated by means of blood pressure and renin activity determination. (2,24)

Childhood	Adolescence/adulthood
Glucocorticoids Initially Hydrocortisone = Hydrocortone® 25-30 mg/m²/d Three daily doses, 50% in the morning Hydrocortisone = Hydrocortone® 10-15 mg/m²/d Three daily doses, 50% in the morning	Hydrocortisone = Hydrocortone® 10-15 mg/ m²/d Three daily doses, 50% in the morning
	Alternative I: Prednisolone 2-4 mg/ m²/d Two daily doses
	Alternative II: Dexamethasone 0,25-0,375/0,5 mg/ m²/d One daily dose in the evening
Mineralocorticoids 9α-Fludrocortison = Astonin H® 0,05-0,10 mg/d Two - three daily doses	9α-Fludrocortison = Astonin H® 0,1-0,15 mg/d Two daily doses

Table 3: Medical treatment before and after completion of growth (10,33), modified..

5.3 Salt substitution

Salt might be given to children with the salt-wasting from, the amount should be from one to two g per day. After the first year of life, salt substitution is not necessary. Patients should be animated to ingest salt in sufficient amounts according to their appetite, probably needing more during exhausting or hot periods in daily life. (2)

5.4 Therapy in infants

Renin activity, electrolyte levels, height and weight need to be controlled constantly. (24)
Treatment in infants has to be accomplished accurately because of higher susceptibility to side effects of cortisone and possibly aldosterone deficiency. More than half of the affected babies are salt-wasters. Low blood sugar levels as a medical problem occur in 9% of all newborn infants. (19)

5.5 Therapy in children

In children, the medication used is hydrocortisone. This substrate is simple to handle, administered as tablet and dosed between 10 to 15 mg/m². One third of the dose should be taken matutinal, the other two thirds vespertine. However hydrocortisone doses need to be adjusted to prevent Cushing's syndrome. (1)
Growth and final height outcome in patients with CAH is often suboptimal, with androgens causing early stop of bone growth and glucocorticoids impairing growth in general.
Some studies mark the first two years and the years of puberty as the most important periods influencing growth, and it is claimed that an early diagnosis and medical treatment have a benefit as well. The amount of glucocorticoids is also an important factor concerning final height outcome. (2)

5.6 Therapy in adulthood

When growth is completed, HC could be changed to prednisone, prednisolone or dexamethasone. The equivalent dose to hydrocortisone is 0,20 – 0,25 in prednisone/prednisolone and 0,03 – 0,04 in dexamethasone. (10)
In adult women with non-classical CAH, androgens must be antagonized. However therapy with glucocorticoids and anti-androgens in women requires caution and frequent medical controls respectively check-ups. CAH treatment also has to be arranged carefully during pregnancy in affected women, for androgens can pass the placenta just like certain glucocorticoids and have a negative effect on the foetus. Therefore, dexamethasone is not used in pregnancy. The glucocorticoids of choice are prednisone and prednisolone, which do not affect the unborn baby. (2)

5.7 Stress dosing

Patients with the classic form of CAH cannot produce sufficient glucocorticoid amounts when substantially stressed, e.g. when being ill, injured or when having a surgical procedure. Daily doses should be doubled or tripled, medication can be administered orally or intramuscular, also by suppositories or by parenteral medication.
Cortisol loss may lead to dangerously low levels of blood glucose. These values should be controlled and carbohydrate intake should be increased. It must be taken into account that physical stress needs higher doses of glucocorticoids. (2,24)
CAH patients should identify themselves as such, and also keep a pass describing their condition and medication. Also, affected individuals respectively relatives should receive HC injections to keep with them in case of emergencies. Relatives must be instructed on medical handling of emergencies (HC injecting). Moreover, dexamethasone is not recommended in stress situations. Mineralocorticoids do not need to be augmented in stress situations. (1)
The non-classic form does not need doubling or tripling if there is no initial gland-suppressing glucocorticoid treatment. (2)

5.8 Therapy of salt-wasting:

Salt wasting needs substitution both of glucocorticoids and of mineralocorticoids. This condition occurs in 21-hydroxylase-disorder, 3β-HSD disorder and the lipoid form.
Fludrocortisone is the administered substrate of choice in affected individuals. It has a good mineralocorticoid effect. Patients should be encouraged to imbibe salt to taste.
Androgens need to be administered in total blocking of the enzyme pathway like in lipoid CAH, and for instance also in 3β-HSD disorder or 17,20-lyase disorder. (1)
The perilous condition of acute salt-wasting is mostly caused by simple febrile infections. Glucocorticoid dose should be augmented three to five times to provide sufficient maintenance levels. Electrolyte imbalances can be treated with special oral rehydration fluids. Moreover, dehydration is accomplished by giving fluids containing sodium chloride respectively glucose. (10)

5.9 Therapy in late onset CAH:

Low–dose glucocorticoid therapy is used in late onset CAH. In childhood, HC is recommended, whereas dexamethasone is used when growth is terminated. These therapeutic measures can help to improve the effects and outcome of non-classic CAH especially in women, concerning hyperandrogenaemic symptoms such as dysmenorrhea or hirsutism. (1,34)

5.10 Therapy control:

The aim of therapeutic intervention is to administer as little as necessary and as much as needed in order to achieve satisfying growth progress and hormonal control. 17-OHP should range between 500 and 1000 ng/dl respectively 12 and 36 nmol/L. Measurement should take place matutinal before glucocorticoid intake. Moreover, suppressed sex steroids (e.g. testosterone, androstenedione and others) in affected individuals are the intended findings in treatment. (1,2)

Testosterone levels can help to evaluate the accuracy of therapy in girls and in boys before puberty. (35)

Plasma renin activity is also a tracer of therapy success, being high in salt-wasting and normal in well-controlled individuals. (1)

5.11 Prenatal treatment

Women are treated with glucocorticoids if there is the possibility that the foetus is suffering from the classic form of CAH. Masculine features in girls begin to develop within the first trimenon. Therefore treatment should be started as soon as pregnancy is attested. CVS and amniocentesis are needed urgently for genetic analysis on CAH. (2)
Therapy should be started as soon as pregnancy is emerged, both in 21-hydroxylase and 11β-hydroxylase deficiency respectively a pregnancy at risk of these disorders. (1)
Dexamethasone is the glucocorticoid used in prenatal therapy to prevent virilisation. It is transmitted from the maternal blood into the foetal blood via the placenta and is not underlying metabolic change or degradation. 0,5 mg of dexamethasone are administered three times a day, and accordingly the dose should be about 20 µg/kg of the mother's usual body weight. (1,2,10)

The medical intervention may also prevent the need for surgical procedures in ambiguous genitalia if administered early and sufficiently. (36)
Dexamethasone administered prenatally has been used for many years now. It shows satisfying repression of masculinization in girls and has no severe adverse reactions according to New et al. Moreover, treatment is non-hazardous both for mother and unborn child. Children prenatally treated with dexamethasone were indistinguishable from not affected children at birth. (34)

A female foetus that is affected is treated throughout the whole pregnancy. Therapy with dexamethasone is abandoned if the medical condition of CAH is not confirmed respectively if the baby turns out to be healthy, or if the foetus is a boy. A male foetus or an unaffected female foetus does not need to be treated. CVS and amniocentesis are essential for the diagnosis. In affected children, dexamethasone therapy can suppress androgen excess sufficiently and prevent ambiguous genitalia. (1–3,10)

Affected female foetuses prenatally treated with dexamethasone show external genitals with no pathologic findings or at least a very low degree of masculinization in 85% when born. However there should be postnatal medical supervision both in affected and unaffected children treated with glucocorticoids during pregnancy. (24)

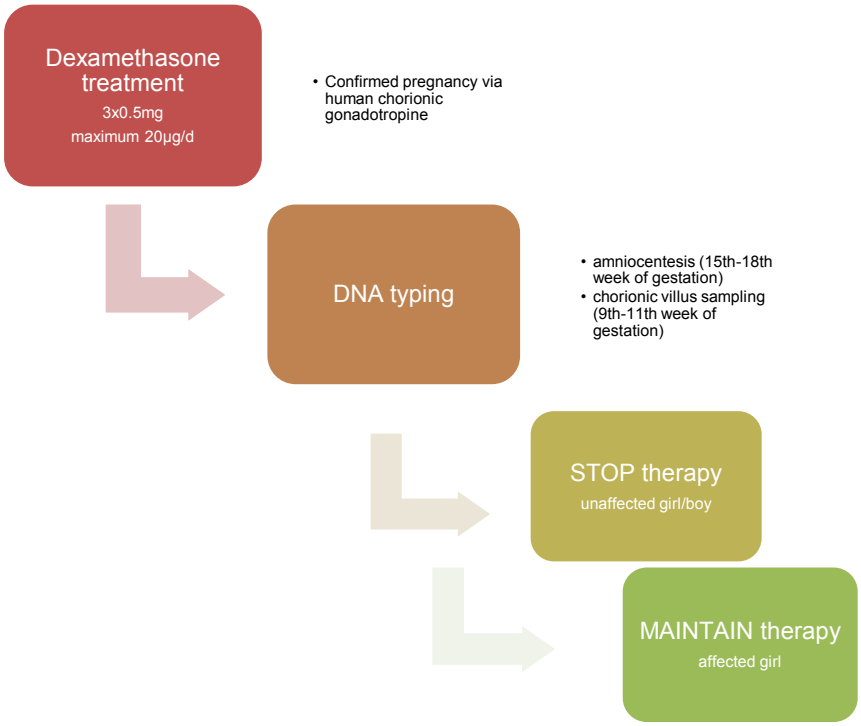

Figure 8: Prenatal diagnosis and therapy (1,10,33), modified.

Prenatal therapy is recommended in individuals possibly affected by classic CAH only and is not indicated in non-classic forms. However treatment during and throughout pregnancy improves the level of genital augmentation and leads to females born with normal genitalia in many cases. (34)

There are no data available on results of long-term side effects of therapy in pregnancy. It has been proposed that various side effects of glucocorticoid therapy can occur in pregnant women, such as weight increase, stretch marks or fluid retention resulting in oedemas. (24)

Therapy in pregnant women with a foetus possibly suffering from CAH is initiated if the following conditions are fulfilled.

1. Sisters/brothers or relatives related by blood suffering from classic CAH diagnosed in DNA testing
2. Assuming that the father of the foetus and of the affected sibling are the same
3. Possibility of gene testing on CAH
4. Commencement of medical treatment within the first nine weeks of pregnancy calculated from last menorrhea
5. No planned ending of pregnancy respectively plan to carry the child to term
6. Sufficient compliance

Table 4: Conditions for prenatal therapy of a possibly affected foetus (24)

Before treatment is started, there should be a genetic analysis of both parents and eventually affected siblings. Moreover there should be a genetic analysis of the foetal DNA concerning 21-hydroxylase affection and a determination of sex i.e. karyotyping of the foetus.
With both parents carrying a defect CAH gene, the risk for a female foetus to be affected is one in four, therefore prenatal therapy is seen critical. One must take into account that the children are treated despite being healthy. There may be side effects of treatment that require further investigation in future studies. Growing may be impaired and mothers risk developing Cushing's syndrome. (10,24,37)
In addition, parents also have to assume responsibility for prenatal dexamethasone treatment, requiring consequent monitoring and both knowledge and handling of risks. (2)

5.12 Future therapy:

Excision of the adrenal gland is an option in individuals not responding to any attempts of whatever treatment. Corticotropin releasing hormone antagonists as a therapy strategy need further studies. Anti-androgens and aromatase inhibitors as an addition to glucocorticoid treatment also require further studies. These methods are all only beginning to come into existence, and further research is needed n order to evaluate the therapeutic benefit. (24)

6 Genetic analysis

Cause of enzyme deficiency in CAH is a mutation, respectively a deletion on chromosome 6q21 in 21-hydroxylase deficiency, on chromosome 8q21 in 11β -hydroxylase deficiency, and in 17α -hydroxylase deficiency, the location of the affected gene is on chromosome 10q24.3. In all three forms, hereditary transmission is autosomal recessive. (14)
Parents of affected patients should be tested for genetic aberrations with regard to further respectively future pregnancies. Depending on the result of amniocentesis and chorionic villus sampling, therapy is induced or not. There is the possibility of the foetus being compound heterozygous, meaning that both the maternal and paternal alleles hold differentiating mutations in the particular place. If this happens, enzyme activity will be decreased to the extent of the milder mutation. Therapy may not be necessary, for example if one parent holds a late-onset mutation whereas the other parent features a severe mutation. Enzyme deficiency will only be present as late-onset deficiency and rest activity may exist still and prevent steroid deficiency. (10)
Therefore, genetic analysis is very important and greatly affects the course of CAH.

7 Management of CAH:

7.1 Management of CAH at birth and in childhood

Female infants born with abnormal respectively enlarged genitals need to be seen by a paediatrician as soon as possible, more precisely by an endocrinology expert with clinical experience in CAH. This is necessary due to medical reasons and to psychological reasons as well. Parents may feel very uneasy about the medical condition of their newborn child and need to get an explanation and support. There is need for complete history-taking, clinical check-up, determination of genetic sex, sonography including the adrenal glands and the urogenital system, and of course a precise determination of 17-OHP. (24)
Patients with CAH should be examined in a specialized clinical institution every six to twelve months. Therapy has to be monitored carefully both to prevent undertreatment and overdosing. (10)

7.2 Management of CAH in adolescence and adulthood

There should be broad medical advice concerning the condition throughout life, for example on the issue of fertility. It is necessary to make clear that fertility and prevention of testicular tumours need adequate medication and compliance, especially in young men. Medical advice is also required on the issue of fertility in women, CAH often having an influence on the unfulfilled wish for parenthood. There should also be regular medical check-ups, e.g. ultrasound, determination of hormones and electrolytes and MR scan for detecting eventual testicle changes. (24)
In adults, there is often the possibility to reduce therapy. However, metabolic issues, weight problems and hypertension may occur due to excessive glucocorticoid and mineralocorticoid dosage. (10)

7.3 Management of CAH during pregnancy

If the mother is affected by CAH, medical treatment should be maintained with non-placenta-passing substrates such as hydrocortisone or prednisolone. Dexamethasone should be avoided due to reaching the foetal circulation by placenta transmission. Caesarean section should be considered if genital correction was performed in pregnant women in order to avoid damages. (24)
If both the mother and the unborn child should be treated, dexamethasone can be used and administered with 20 µg/kg/d in three single doses. (33)

8 Diagnosis

There are three ways of determining CAH: by phenotypic features, by measurement of hormone levels and by detection of genetic alteration in the analogous gene locus. (1)

8.1 Diagnostic parameters

8.1.1 17-hydroxyprogesterone

As the hormonal pathway is blocked, hormonal precursors accumulate. A typical marker for CAH is 17-OHP. Levels are higher in salt-wasting patients than in non-salt-wasting patients. Neonatal screening in many countries includes measurement of 17-OHP, although results may not be convincing in pre-term born infants. (2)

In CAH, hormone levels show decreased end products and elevated pre-stages. Classic CAH shows little cortisol, ranging from low to regular levels, while 17-OHP and androgens will be high. Especially 17-OHP can be found augmented many times from basic level. (38)

Non-classic CAH may show unremarkable 17-OHP values. However, one cannot rely on 17-OHP levels only as a diagnostic tool in non-classic CAH for values may be in normal range, although determination in the morning may be the most reliable one. Moreover, a quantification of 17-OHP in the early morning saliva is also possible, showing appropriate correlation with findings of quantification in serum. (39)

8.1.1.1 17-OHP in infants

17-OHP is measured in newborn screening nowadays. Basically, classic forms can be detected and non-classic forms are unlikely to be detected. (22)

For better detection of affected individuals and better prevention of finding false-positives and false-negatives, the range of 17-OHP levels is matched with gestational age and birth weight nowadays. Especially in premature infants, interpretation of 17-OHP is not reliable respectively valid due to physiologically elevated 17-OHP concentrations. However, gestational age is even more reliable than body weight. (40)

Moreover, results of measuring 17-OHP depend on the technology used and therefore international levels in different countries cannot be compared without making adjustments. In some countries, there is an emphasis on gestational age in evaluation of 17-OHP, whilst the emphasis is on birth weight in others. The US, Canada and New Zealand rely on birth weight to evaluate 17-OHP levels in infants, while in Europe and Japan gestational age is more common. (40,41)

BW [g]	Age [d]	17-OHP values [nmol/liter]		
		Normal	CAH possible	CAH probable
> 2500	1 – 2	< 60	60 – 90	> 90
	3 – 4	< 40	40 – 90	> 90
	> 5	< 30	30 - 90	> 90
2,000 – 2,500	1 - 2	< 60	60 - 125	> 125
	3 – 4	< 50	50 - 125	> 125
	5 - 14	< 40	40 - 125	> 125
	> 15	< 30	30 - 125	> 125
1,500 – 2,000	1 - 4	< 80	80 - 150	> 150
	5 - 14	< 60	60 - 150	> 150
	15 - 30	< 40	40 - 150	> 150
	> 30	< 30	30 - 125	> 125
1,000 – 1,500	1 – 4	< 150	150 - 200	> 200
	5 - 14	< 120	120 - 200	> 200
	15 - 20	< 80	80 - 200	> 200
	21 - 30	< 60	60 - 200	> 200
	31 - 60	< 40	40 - 125	> 125
	> 61	< 30	30 - 90	> 90
< 1,000	1 - 20	< 200	200 - 300	> 300
	21 - 30	< 100	100 - 200	> 200
	31 - 60	< 60	60 - 150	> 150
	> 61	< 30	30 - 90	> 90

Figure 9: 17-OHP cut off levels adjusted for weight and age
adapted from Olgemoller, JCEM 2003

8.1.1.2 17-OHP in older children, men and women

In older children, adolescents and adults, 17-OHP levels should be in the following range, according to the Hormonlabor der Univ.-Klinik für Frauenheilkunde und Geburtshilfe Graz.

17-OHP levels	men		0,2 – 2,3 ng/ml
	women	Follicular phase	0,2 – 1,3 ng/ml
		Luteal phase	1,0 – 4,5 ng/ml
		menopausal	0,2 – 0,9 ng/ml
	children		0,2 – 0,9 ng/ml

Table 5: Normal range of 17-OHP in serum/plasma
adapted from: Hormonlabor der Univ.-Klinik für Frauenheilkunde und Geburtshilfe Graz

8.1.2 ACTH test

A common way to examine an underlying CAH condition is the application of ACTH and measurement of 17-OHP and cortisol approximately one hour before and one afterwards. 0,125 or 0.25 mg of ACTH are administered. (5)

In normal individuals, there should be no significant elevation of 17-OHP, whereas in affected individuals there is an afflux of the substrate. 17-OHP levels under 30 nmol/L exclude an underlying CAH, whereas affected persons reach17-OHP levels over 45 nmol/L after ACTH administration. (27)

Basically, 17-OHP may be used as a marker in screening, but administering ACTH is both more sensitive and specific. Moreover, it is more likely to find non-classic forms in the ACTH test than by standard 17-OHP measuring. (2,42)

If CAH is suspected in infants, the following possibilities should be taken into account:

Diagnosis in neonates:
1. Search for genetic aberrations and a karyotype testing in children with undefined genital findings
2. Application of ACTH in infants and measurement of 17-OHP and cortisol before and afterwards. This should not take place within the first day of life, showing typically high hormone levels and leading to false conclusions.
3. Measurement of mineralocorticoids and renin activity within the ACTH application
4. Screening of urine for sodium and potassium excretion
5. Quantification of the decline of formation of steroidal products by application of cortisol

Table 6 : Finding a diagnosis in infants with suspected CAH (1), modified.

8.2 Diagnostic measures in other forms of CAH

8.2.1 Non-classic CAH

The non-classic form of CAH is diagnosed in an ACTH stimulation test measuring 17-OHP levels one hour before and one hour after the application of an ACTH bolus. Non-classic forms may show normal levels of 17-OHP without stimulation, therefore further tests are necessary and simple 17-OHP plasma levels are not reliable. (6,27)
However medical therapy is only needed if there are clinical symptoms. Frequent manifestations of non-classic CAH include impaired fertility, dysmenorrhea, increased bone maturity, decreased growth, masculine hair-growth in women and serious acne. (2,24)

8.2.2 11β-hydroxylase

11β-hydroxylase is involved in the mineralocorticoid formation as well as in the glucocorticoid formation. Besides 21-hydroxylase deficiency, it is the second most common form of CAH. Non-classic forms may be more frequent than classic 11β-hydroxylase CAH, however there are no data on frequency. (25,43)
Deoxycorticosterone and 11-deoxycortisol reach a high level in serum analysis. Moreover metabolites of the named substrates are elevated in the excreted urine. 11-deoxycortisol is not transformed sufficiently into cortisol and pre-stages are shifted into the formation of aldosterone. Furthermore, the ACTH stimulation test is also a possible diagnostic tool, and of course genetic testing can be obtained. (1)

8.2.3 3β-hydroxysteroid-dehydrogenase

In case of dysfunction, pregnenolone, 17-OHP and DHEAS will accumulate. Moreover, there is more pregnentriole and 16-pregnentriole to be found in the urine. A further diagnostic tool is the 60 minutes stimulation test with ACTH. There is a classical and a non-classical form, the latter one being less serious respectively showing fewer symptoms. The classic form can be incompatible with life due to serious lack of cortisol. (1,44)

8.2.4 17-α-hydroxylase disorder and 17,20-lyase disorder

17α-hydroxylase transforms pregnenolone into 17α-hydroxypregnenolone and progesterone into 17-OHP. 17,20-lyase turns 17α-hydroxypregnenolone into DHEAS and 17-OHP into androstenedione. Disorders within these enzymes can occur separately or in combination, whereas the combination appears more frequently. Basically, there is a lack both of androgens and glucocorticoids. Diagnostic features can be elevated blood pressure (in children) with low potassium and mineralocorticoid levels in 17α-hydroxylase deficiency. In 17,20-lyase deficiency there may be underdeveloped genitalia and missing pubarche due to lack of androgens. (1,45)

8.2.5 Lipoid CAH

This uncommon form of CAH is the most severe variation of CAH. No steroids can be formed out of cholesterol. There is poor survival due to lack of mineralocorticoids, glucocorticoids and also androgens resulting in various symptoms, e.g. electrolyte imbalance, salt-wasting etc. (30,46)
There are no steroid products detectable both in blood or urine, and there is an elevated ACTH and plasma renin activity due to lack of functioning of the adrenal gland. Moreover there are mostly feminine but underdeveloped external genitalia in infants. (1)

8.3 Prenatal diagnostic measures

The risk to be born with CAH in a family with affected siblings is one in four due to the autosomal-recessive heredity transmission. The possibility of being affected results from both parents being heterozygous for a CAH mutation. Therefore 25% of descendants of these couples are affected, 25% are healthy, and 50% receive carrier status. (47)

Nowadays, genetic analysis of the 21-hydroxalse gene is used to determine whether CAH is existent in the foetus or not. The foetal cells are won by amniocentesis or CVS, but the time of realisation is still too late to start therapy, which must be administered within the first two months of gestation respectively under the gestational age of 2 ½ months. (34)

Classic CAH	SW 21-hydroxylase	SV 21-hydroxylase	11β-hydroxylase	17-hydroxylase/ 17,20-lyase	3β-hydroxysteroid dehydrogenase	Lipoid CAH
Gene defect	CYP21A2	CYP21A2	CYP11B1	CYP17	3β-HSD 2	CYP11A
Gene locus	6p.q21	6p.q21	8q21	10q24.3	1p13.1	15q23-24 8p11.2
Ambiguous genitalia	In girls	In girls	In girls	In boys	In boys	In boys
Salt-wasting	Yes	No	No	No	Yes	Yes
Blood pressure	↓	±	↑	↑	(↓)	↓
Serum sodium levels	↓	±	±, (↑)	±	↓	↓
Serum potassium levels	↑	±	±, (↓)	±	↑	↑
Plasma renin	↑↑	±, (↑)	↓	↓	↑	↑↑
Glucocorticoid levels	↓	↓	↓	↓	↓	↓
Mineralocorticoid levels	↓	±	DOC↑ Aldosterone ↓	DOC↑ Corticosterone↑	↓	↓
Androgen levels	↑	↑	↑	↓	↓	↓
Steroid profile	17-OHP ↑↑ 21-DOC ↑↑ Testosterone ↑ Androstened. ↑ Aldosterone ↓	same	DOC ↑↑ 11-DOC ↑ Testosterone ↑ Aldosterone ↓	Progesterone↑ Deoxycorticost. ↑ □□□□□□□□□□□□□□ ↑ □□□□□□□□□□□□↓ C□□□□□□□↓ □□□□□□↓	DHEAS ↑ Pregnenolone ↑ 17-OH-Pregnenolone ↑	↓↓

Table 7: CAH clinical and laboratory parameters (10), modified.

8.4 Control parameters

Control of CAH should include determination of electrolytes, renin activity, glucocorticoids and precursors, e.g. 17-OHP, mineralocorticoids and precursors as well as sex steroids and precursors, e.g. androstenedione or testosterone in serum/plasma. These controls need to be done every three months in infants and approximately every four months up to one year in older children. Other possibilities of monitoring are salivary samples, blood spots like in neonatal screening and the measurement of excreted electrolytes in the urine. (24)

9 Screening programme

Since 1966, neonatal screening for hereditary disorders concerning metabolism and endocrinology is performed in newborn children in Austria. The range of disorders was augmented within the years, now including screening for diseases like cystic fibrosis, galactosaemia, hypothyreoidism, biotinidase deficiency, phenylketonuria, congenital adrenal hyperplasia and more. Tandem mass spectometry is used in screening since 2002, augmenting the range of possibly detectable disorders. (48)

Screening for 21-hydroxylase CAH by measuring 17-OHP levels finds both affected boys and girls, whereas diagnosis in boys used to take place later in life before the introduction of screening due to lacking of ambiguous external genitalia at birth. (24)

9.1 Sample taking and analysis

17-OHP is measured in whole blood dried on filter paper. The blood sample is taken between the second and third day of life. As gestational age and birth weight influence the 17-OHP level, there are cut-off levels according to these factors. Adjustment is important to prevent false positive or false negative results, for in pre-term infants levels may be higher and therefore contort correct diagnosis. (40,48,49)

The following table shows range of pathologic 17-OHP levels according to gestational age.

Gestational age	17-OHP level nmol/l
36 and beyond	40
35	60
34	65
32	80
30	120
28	300

Table 8: 17-OHP levels requiring a second sample (49), modified

The screening cards are sent to the National Newborn Screening Laboratory in Vienna for analysis. The samples need to get to an accurate laboratory in time respectively without delay, otherwise false results may distort the diagnosis. If there is a pathologic profile, a second analysis is performed before concrete diagnosis is claimed. In case of a second positive result, the responsible centre respectively medical contact person is informed as soon as possible in potentially dangerous disorders that could lead to a metabolic crisis in the newborn infant. In case of doubt, other hormonal values need to be determined in addition as well, e.g. various mineralocorticoids, androgens and glucocorticoids. Nevertheless further testing is initiated to confirm the diagnosis in the specific centres, such as Graz or Salzburg. (48)

Three options are used to test CAH worldwide: radio-immunoassay in the US, enzyme-linked immunosorbent assay in Japanese children, and time-resolved Fluor-immunoassay in European children. Results respectively 17-OHP levels may depend on the option used and must not be compared directly. (40)

9.2 Benefits of newborn screening

The benefit of screening is to prevent perilous effects of CAH in the newborn, to treat early, and to guarantee correct sex assignment. Screening leads to an earlier diagnosis, fewer stays in hospital, and fewer deaths due to salt-wasting in newborn children. The introduction of screening possibly improves course and prognosis oft the disease and minimizes consequential damages. (40,48)

9.3 Screening results worldwide

21-hydroxylase-deficiency seems to occur as often as 1 in 14,199 newborn children featuring homozygous alleles all over the world, and 1 in 60 newborn children featuring carrier status respectively heterozygous alleles. In Caucasians, 1 in 11,909 individuals may be affected, and 1 in 55 may have carrier status. The salt-wasting form is much more frequent than the non-salt-wasting form, the ratio being three to one. There are two populations that were found to have a higher prevalence of 21-hydroxylase deficiency in comparison to other populations all over the world, including the Alaskan Yukip eskimos and the population of the former French colony island La Réunion. The former have a prevalence of 1:282, the latter a prevalence of 1:2,141. In Italian and French children, there were more cases of CAH than in Scottish and New Zealandan children. In Japanese children, CAH frequency is similar to Caucasians. (13)

In 13 countries, neonatal screening for CAH is provided nowadays, including the US. (50)

Screening cannot guarantee to find every affected individual. In some cases, especially milder and late-onset forms, diagnosis will have to be made according to clinical features. Moreover, 3β-HSD and 17-hydroxylase/17,20-lyase defect are not detected in newborn screening for congenital hyperplasia. (49,51)

In both classic and non-classic 11β-hydroxylase deficiency, there may also occur normal or only slightly elevated hormone levels. (52)

In addition, the aim of screening is to identify 21-hydroxylase deficiency in the first place, which is rather frequent. 11b-hydroxylase deficiency is not as frequent and may not be as severe. (53)

10 Health problems in CAH

10.1 Growth

A lot of people suffering from congenital adrenal hyperplasia reach an adequate height due to good medical treatment and supervision. It has been shown that height outcome is better than generally thought, with height decrease not as severe as expected. (24)

There are various studies on height in affected patients. Many of them reach an adequate stature under continuous medical treatment, whereas others do not achieve target height. Influences on outcome may be numerous and are not analysed to detail, but may include discontinuing therapy, too low-dosed or high-dosed therapy, insufficient response to medication etc. (1)

Longitudinal growth seems to be affected both in untreated and in adequately treated patients, the latter being closer to target height, but also not reaching a sufficient final height outcome in many cases. (10)

One study by Bonfig et al. found that salt-wasters were diagnosed as early as 0.3 years, whereas non-salt-wasters were only diagnosed at 2.2 years on average. Girls with the severe, salt-wasting form had a better height outcome than girls with the non-salt-wasting, simply virilising form. Boys did not differ in height outcome, no matter what form they were suffering from. Moreover, it was discovered that adult CAH patients were not as tall as the healthy comparison group. (54)

In another study by Manoli et al., height in patients with the SW from was not significantly distinguishable from expected height calculated from parental height, whereas it was significantly decreased in patients with the SV form. In SV patients, there is a much more negative effect of the disorder on their height and they often do not reach expected target height, moreover advanced bone age and precocious puberty occur more often within these individuals. (55)

In a study by Eugster et al., it turned out that the final height of CAH patients suffering from 21-hydroxylase deficiency is frequently within one standard deviation from target height. In addition, probands were divided into a well controlled and a poorly controlled group reflecting on compliance, and it emerged that growth is better in well controlled subjects. Furthermore, early diagnosis also accounts to optimal final height. (56)

10.1.1 Medical treatment and final height

In the study by Eugster et al., hydrocortisone has a better outcome in final height than prednisone, and prednisone does not have a better effect than hydrocortisone on sex steroid suppression. The HC dosing in early childhood (children younger than 2 years of age) did not have an influence on tallness in adulthood, whereas dosing in adolescence had a significant effect. Dosing greater than 20 mg/m² per day decreased final height outcome, whereas dosing beyond this level showed a better outcome. Moreover it is claimed in this study that prednisone has a negative effect on the final height outcome, which is not proved in other studies. It is also postulated that prednisone treatment in childhood lowers final height and therefore should not be administered before adolescence. (56)

10.1.2 Sensitive periods in medical treatment

The first two years of life and the pubertal period are the most sensitive periods concerning medical treatment and its influence on final height outcome. Especially the time of pubertal development is a very sensitive time span requiring adequate adjustment of medical treatment in order to provide sufficient hormonal substitution and enable appropriate growth. Nevertheless pubertal growth gain and tallness was lower than in healthy teenagers both in the SW and SV form affecting both sexes at an indistinguishable extent. (54,55)
Nike et al. found out that specifically the periods between six to twelve months of life and eight to fourteen years of life are the most vulnerable timespans having negative effects on growing and tallness in CAH patients. Correlation respectively movement towards correlation between height and administered glucocorticoid dosing has been found. (57)

10.2 Body composition

Not many studies investigate increased weight issues in CAH. However it has been found that elevated BMI is more frequent in affected individuals than in healthy subjects according to Volkl et al. The expected frequency of a deviant BMI estimated to be 2.27% was found to account for 16.8% in the studied CAH group. Moreover, it does not make a difference if patients suffer from the severe or the mild form of classic CAH concerning BMI levels, and sex or age are also no distinctive features. However obesity is more common among children or adolescents descending from overweight parents. (58)

In another study by Cameron et al., BMI levels in affected individuals did not deviate from those in not affected persons. (59)

10.2.1 Medical treatment and BMI

In a study by Stikkelbroeck et al., BMI was not significantly dependent on glucocorticoid therapy and dosage at any age. (57)

It is claimed that the kind of glucocorticoid used for hormonal substitution does not influence the BMI level. No significance was found for dexamethasone, prednisone or hydrocortisone to prevent or provoke obesity. However the amount of glucocorticoids administered, not the kind of glucocorticoid, is an influential factor on BMI levels in affected patients. Mineralocorticoids such as fludrocortisone do not cause obesity. (58)

In a cohort from the UK, BMI levels were elevated and height was decreased in CAH patients in general. It has been proofed that CAH also affects metabolic activity and causes severe health problems. In 41 per cent, patients were obese. 46 per cent had too high cholesterol levels, 29 per cent were inert to insulin, and bone mineralization was impaired at a various extent in 47 per cent. Moreover, reproduction was affected as well in these patients. (60)

10.3 Bone mineral density

Despite the intake of medication such as glucocorticoids and mineralocorticoids, CAH may cause short stature and inhibit linear growth in CAH patients. Compared to healthy individuals, the affected persons were smaller on average. However no harm in bone mineral density was found and values were comparable to unaffected persons, and in addition long-term glucocorticoid treatment may not have a negative effect on bone composition. (59,61)

No difference was found both between the late-onset and severe form of CAH concerning mineralization, and between the affected group in comparison to the healthy control group. Moreover, dosage of glucocorticoids and duration of administration do not influence mineralization as well. (61)

10.4 Bone age

Greulich and Pyle created an atlas to compare radiologic features of patients with standard x-rays of coevals in order to find pathologic alterations. (62)

In congenital adrenal hyperplasia, both tallness and bone age are the most important indicators of successful therapy. As androgens lead to advanced bone age, bone maturity correlates with sex steroid levels. Therefore, children with forms of CAH where sex steroids are increased tend to have advanced bone age, whereas children with the rare CAH forms resulting in lack of sex steroids show diminished bone maturity. Younger children with CAH may show fast growth, but short stature in adolescence respectively adulthood due to early closing of epiphyseal plates. In children with CAH, bone age should be checked once a year on a regular basis. (63)
In newborn babies respectively in infants, bone maturity is not affected yet. (64)
To evaluate bone age in children, x-ray of the left hand is used. The degree of epiphyseal closure, the width of epiphyseal plates and structure of carpal bones are compared to standard x-rays of children of the same age and sex. (65,66)
Moreover, there should not only be a comparison with x-rays of coeval children, but also with neighbouring age brackets in order to find the x-ray that distinguishes the least. (66)
It was found that bone age is advanced the most in male affected patients suffering from the non-salt wasting, simply virilising form, most probably due to late diagnosis and therapy. In addition, pubertal onset was earlier in these boys, compared to the salt-wasting form and healthy individuals. (54)

In another study with children suffering from 11β -hydroxylase deficiency, body composition was also marked by a rapid growth spurt between the 6^{th} and 12^{th} month of life, and a rise of bone age whilst diagnosis had not been confirmed yet. Final height was significantly decreased in all patients, independent from time of diagnosis and therapy control. (26)

10.5 Blood pressure

Higher systolic blood pressure was reported in more overweight respectively obese patients compared to non-obese patients according to a study by Volkl et al. However, patients with normal weight showed a significantly lowered diastolic blood pressure profile. (67)

10.6 Fertility

It is commonly known that both classic as well as non-classic forms of CAH may lead to decreased fertility both in affected men and women.

10.6.1 Women

Females suffering from CAH show decreased fertility rates, fewer pregnancies and fewer births. The more fatal the enzyme deficiency in an affected woman is, the fewer children she usually has. Psychosocial issues may also have an influence on reproduction in affected women. Factors influencing the low fertility or pregnancy rates among women with CAH may also include fewer relationships, less sexual intercourse and limited vaginal penetration due to genital anatomy within affected females. Adequate medical treatment and genital reconstruction can improve fertility rates. (68,69)

In the non-classical form, impaired fertility is less severe than in the classic form. However, glucocorticoid treatment can improve reproduction rates within these women, regulating ovulation and dysmenorrhea that may be the causing factors. (70)

Moreover, polycystic ovary syndrome impairing fertility is rather common in congenital adrenal hyperplasia. Hague et al. found out that PCO occurs in about seventy-six per cent in female patients who already passed their first menstruation. (71)

10.6.2 Men

In men, there is also the possibility of infertility, which may be caused by ectopic adrenal tissue in the testes. These so called TARTs, short for testicular adrenal rest tissue, occur in 45 per cent of CAH patients and more likely in poorly controlled ones. Moreover, sperm analysis is pathological in many cases, occurring more often in non-compliant patients than in well-treated ones. (72)

Another study by Falhammar et al. found that sperm analysis is pathological in 43 per cent and TARTs (so-called testicular adrenal rest tumours) occur in 86 per cent of patients screened in the study, hence impairing fertility among males affected by congenital adrenal hyperplasia. (73)

11 Outcome

Sufficient medical treatment in both male and female patients affected by any form of CAH is illustrated not only by adequate hormonal suppression respectively substitution, but also by physical development, adequate onset of pubertal development and growing progress. (1) Patients suffering from congenital adrenal hyperplasia have no impaired life expectancy due to their condition. However, final height is often impaired even if there is adequate treatment and supervision. Moreover, fertility restrictions are quite frequent, as well as testicular rest tumours. These count among the most distinctive difficulties still unsolved in CAH. (27) Since there are adequate therapy strategies (dexamethasone, hydrocortisone etc.), prognosis has improved significantly for patients suffering from any form of CAH. Management of CAH should aim at soon diagnosing, early medical treatment, reasonable surgical treatment, reaching of target height and urging the patients to a compliant behaviour in general. (24)

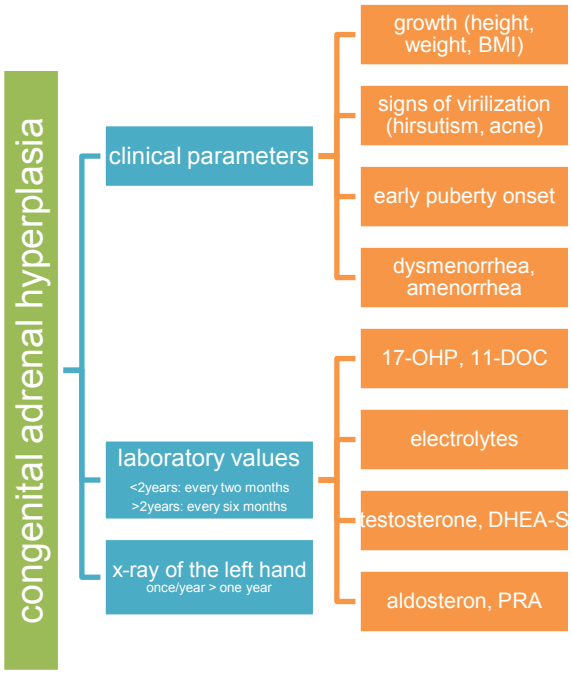

Figure 10: Therapy monitoring and control in CAH – important parameters (33), modified.

12 Material and methods

12.1 Patients

In our retrospective study, we included children that were born in Styria between 1992 and 2011 and were diagnosed with any form of CAH. The cohort included sixteen patients with 21-hydroxylase deficiency and two patients with 11β-hydroxylase deficiency.
Data were extracted from the medical records of the patients treated at the Universitätsklinik für Kinder- und Jugendheilkunde Graz. For this intent, an application was presented to and allowed by the ethics committee of the Medical University of Graz.
The children were treated and data were recorded on a regular basis at the Universitätsklinik für Kinder- und Jugendheilkunde Graz. The age of the patients ranged from infant to 16-year-old when data were collected and analysed.

12.2 Collected data

To evaluate diagnosis, treatment and course of disease in patients with CAH, we utilized several parameters.

12.2.1 Epidemiology and diagnosis

We investigated the time of diagnosis and the reason for suspected CAH. Moreover, we compared diagnosis finding before and after newborn screening was introduced in Austria.

12.2.2 Screening

Children born in Styria, Austria, between 1992 and 2001 (not screened) and between 2002 and 2011 (screened) were included into the study. The numbers of patients with CAH were compared. In **Group A** (patients not screened) CAH was diagnosed by typical signs/symptoms and confirmed by standard laboratory data before age 18 or before end of 2011. In **Group B** 98,7 % of all newborn infants were screened by measuring 17-OH progesterone (17-OHP) in a dried blood spot on filter paper. Diagnosis was then confirmed by standard laboratory methods. Data on screening results were collected at the Labor für Stoffwechseldiagnostik at the department of paediatrics and adolescent medicine at the AKH Vienna.

12.2.3 Virilisation

The degree of virilisation was determined and categorized into Prader stages I-V by evaluation of external genitalia in female patients

12.2.4 Genetics

Seventeen patients were analysed genetically, in one patient analysis had not been available respectively performed. The analysis was performed to detect genetic variations such as deletions, inversions, point mutations, etc. Not only the index patient was analysed, but also both parents and siblings in order to gain valid data on the genetic profile of the affected child.

12.2.5 Familial prevalence

Frequency of CAH in families was investigated and the existence of affected elder or younger siblings was explored.

12.2.6 17-OHP-levels and hormone levels

To evaluate 17-OHP-levels and hence therapy control, the long-term medical records respectively laboratory results of the endocrinology department of the Universitätsklinik für Kinder- und Jugendheilkunde Graz were used. Furthermore 17-OHP screening levels at birth recorded at the Labor für Stoffwechseldiagnostik at the AKH Vienna were compared.
The percentage of normal laboratory hormonal values was compared to pathological values, and mean 17-OHP levels in the single patients were calculated and compared.

12.2.7 Height and weight

To analyse long-term weight and height development, percentile figures and medical records with the collected data from the children's endocrinology department at the Universitätsklinik für Kinder- und Jugendheilkunde Graz were used. The data were collected from birth on up to 2011 in those patients diagnosed early, and from time of diagnosis up to 2011 in the others.
Within the percentile charts, the 3^{rd}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th} and 97^{th} percentile are marked. The median of height and weight in a coeval group of children is the 50^{th} percentile. The 25^{th} and 75^{th} percentile mark one standard deviation from median height respectively weight, and the 3^{rd} and 97^{th} percentile tag two standard deviations. With these charts, height and weight development in the affected patients were analysed. Moreover, BMI was calculated for the CAH probands and compared to coevals. BMI was calculated through kg/m^2. Finally height, weight and BMI SDS as well as height and BMI percentile were calculated and compared.

12.2.8 Bone age

Bone age was determined once a year in most patients and evaluated via the Greulich and Pyle method. (62)
Bone maturity was analysed and mean advanced respectively diminished bone maturity was calculated and compared among the probands.

12.2.9 Medication

Medical treatment was researched in the medical records and the mean dose was calculated and compared among the probands.
All patients were treated with glucocorticoids (Hydrocortone®) when diagnosed with CAH, and some were treated with mineralocorticoids (Astonin H®) in addition. Most patients were treated from birth on and therefore more data were available. Five patients were treated later due to delayed diagnosis.

12.3 Statistical evaluation

The programmes that were used to calculate are Microsoft Excel 2011® (Mac) and KIGS Auxology Calculator, Pfizer Company 2008®.

13 Results

From 1992 to 2011, a total number of 18 patients were found to suffer from congenital adrenal hyperplasia in Styria.
Six boys and twelve girls were diagnosed, which does not comply with the 1:1 gender ratio found in literature. (3)

13.1 Epidemiology

The prevalence of CAH differs considerably all over the world and was not exactly known for our region respectively in the Austrian population.
A number of patients with CAH might not be diagnosed, especially males. CAH occurs due to a defect in the 21-hydroxylation ('classical CAH') in about 95 % of all cases. (18)

Figure 11: Styria/Austria, Population 1.213.255 (2011) (74)

Newborn screening for CAH, based on the measurement of 17α-hydroxyprogesterone (17-OHP) was shown to be efficient for diagnosis, and is part of the newborn screening programme in Austria since April 2001.

In our study we compared 2 groups of children:
Group A were children born in Styria (a province of Austria), 1992 – 2001, n = 119.001, m 61.256, and f 57.745. Group B were children born in Styria 2002 – 2011, n = 103.228, m 52.722, and f 50.506. (75)

In group A (patients not screened), CAH was diagnosed in six children (m 3; f 3); two of them with simple virilising (SV) 21-OH deficiency (m 1; f 1) and four with salt wasting (SW) 21-OH deficiency (m 2; f 2), with a calculated prevalence of 1:19.833 in this group (SW and SV CAH).

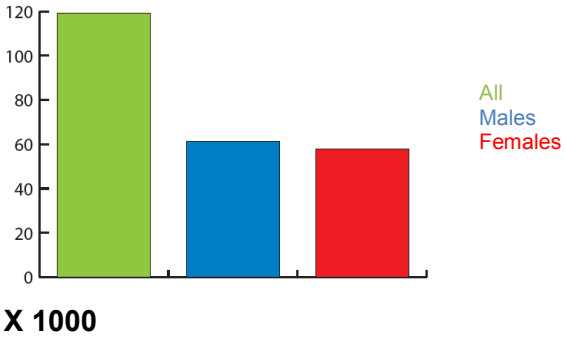

Figure 12: Group A, born 1992-2001

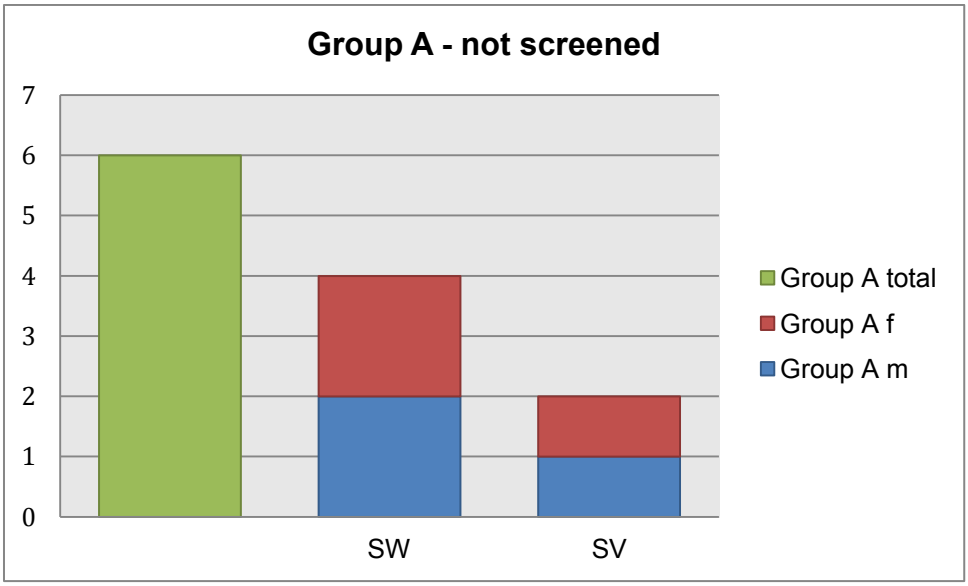

Figure 13: Group A, CAH, not screened

In group B, CAH was diagnosed in twelve children (m 3; f 9). Eight of them were diagnosed with SW 21-OH deficiency (m 2; f 6), two with SV 21-OH deficiency (m 0; f 2) and two with 11β-hydroxylase deficiency (m 1; f 1). Calculated prevalence of CAH due to SW 21-hydroxylase deficiency was 1:12.903 in this group. Prevalence of SV 21-OH deficiency and SW 21-OH deficiency together was 1: 10.322, and prevalence of11β-hydroxylase deficiency was 1:51.614.

17-OHP levels were measured in children at birth. Not all of them showed elevated 17-OHP levels. One girl was diagnosed not at birth but within the first year of life due to genital ambiguity and one girl was diagnosed at the age of three due to pubertas praecox. Another girl had already been diagnosed prenatally due to affected elder siblings.

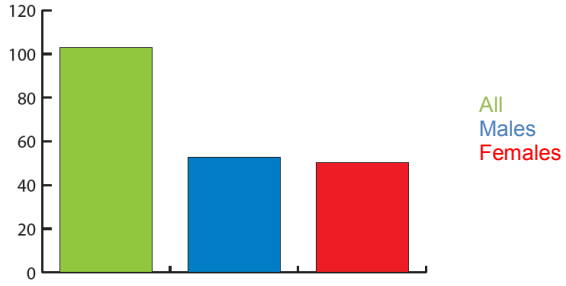

Figure 14: Group B born 2002-2011

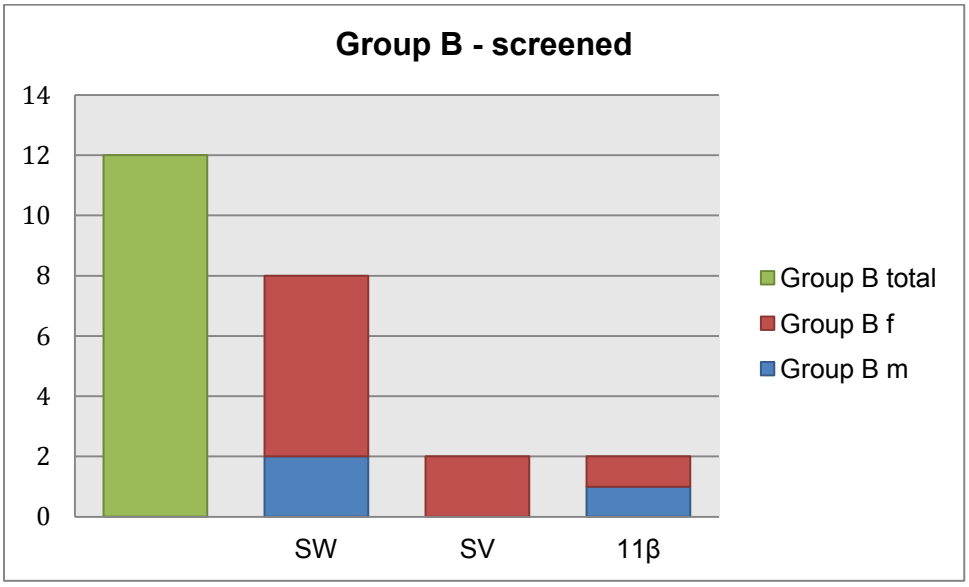

Figure 15: Group B, CAH, screened

Whereas group A displayed the expected sex ratio, group B showed a strong female predominance (m 3; f 9).
These data show that newborn screening for CAH seems to increase the rate of detection of CAH.

13.2 Diagnosis

From eighteen children in our cohort, twelve were screened for CAH at birth. However, in three of the affected children the condition was not detected immediately. The boy with 11β-hydroxylase deficiency (who had only slightly elevated 17-OHP levels) and the girls with SV 21-hydroxylase deficiency (who had normal 17-OHP levels) were diagnosed due to genital ambiguity, pubertas praecox and growth spurt at the age of one, two respectively three.
From the six children who were not screened, two girls were diagnosed due to ambiguous external genitalia. One boy was diagnosed due to rapid growth spurt at the age of six, and another due to pubertas praecox at the age of seven. Two children were found to have CAH due to affected elder siblings and consecutive exploration.

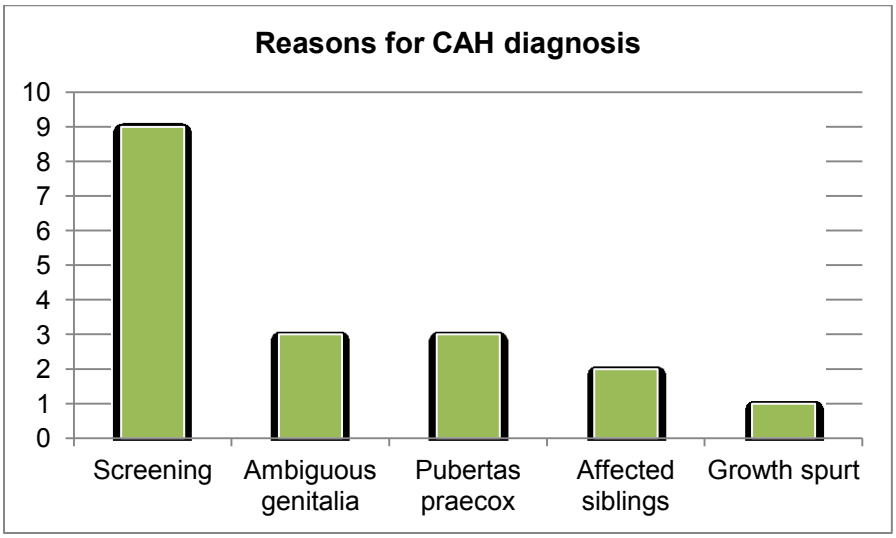

Figure 16: Reasons for CAH diagnosis in the 18 affected children in our cohort

13.3 Virilisation

Girls diagnosed with CAH showed ambiguous external genitalia at a various extent. Only one girl showed no pathological findings when born. Three girls showed Prader I virilisation, one girl Prader II, one girl Prader II-III, one girl Prader III, three girls Prader III-IV, one girl Prader IV and one girl Prader V. The grade of ambiguity varied significantly among the female patients, both among the screened and the not screened children. However the most severe Prader V was only found in one girl that was born before screening was introduced.

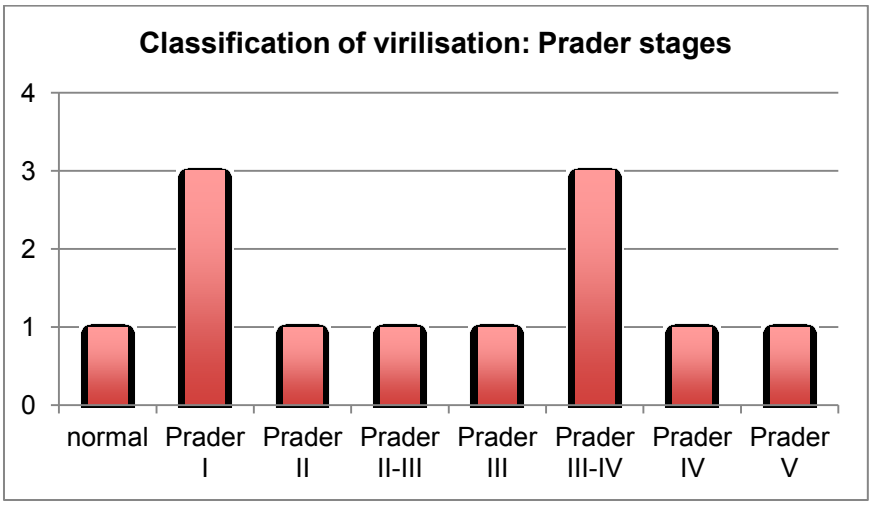

Figure 17: Frequency of Prader stage I-V among Styrian female patients

13.4 Genetics

As little as ten mutations account for more than 90% of all 21-hydroxylase deficiencies. (27) In our cohort, most patients were compound heterozygotes. This implies that they inherited two different, pathologically transformed alleles from both parents, resulting in the CAH form of the lesser degree. In one patient, there was a homozygous deletion in the 21-hydroxylase gene. In another patient, there was a heterozygous defect, however the father had no detectable mutation on the 21-hydroxylase gene. Genetic analyses of the fifteen other patients (thirteen with 21-hydoxylase deficiency and two with 11β-hydyroxylase deficiency) showed that both parents were heterozygous for a mutation in the 21-hydroxylase respectively 11β-hydroxylase gene, hence being asymptomatic carriers. In one case, the results of genetic analysis were not available.

13.5 Familial prevalence

As CAH is an autosomal recessive disorder, it can only occur when both parents are carriers, and only in 25% of descendants, following Mendel's laws. (8)
It is interesting to report that many of the affected individuals in our cohort have affected siblings, and that CAH occurs more often within these families than expected.
In one case, there was a family with four children in our cohort, where all of the children suffer from salt-wasting CAH and genetic analysie show the same mutation in the concerning gene locus. From the twelve remaining children with 21-hydroxylase deficiency, another two are siblings, and at least three of the other children have elder or younger siblings (thus not being in our 1992 – 2011 cohort) who are affected.
The two patients in our cohort suffering from 11β-hydroxylase deficiency are also siblings and are carrying the same mutation according to genetic analysis.

13.6 17-OHP levels in screened infants

In Austria, 17-OHP levels are adjusted both to birth weight and gestational age. There is a cut-off level table categorizing the measured 17-OHP levels into normal, CAH possible and CAH probable.
Screening was introduced in April, 2001. Therefore twelve of the CAH patients who were born afterwards have been screened. Screened 17-OHP levels in affected children ranged from 26.6 nmol/l to 890 nmol/l.
Summing up, two infants presented levels for CAH possible, and seven children levels for CAH probable. One child was not diagnosed at birth due to screening 17-OHP level (respectively 17-OHP was not listed) but later on due to early pubarche. From another two children with normal levels, one child was diagnosed in early childhood by a paediatrician due to genital features, and one girl was already diagnosed at birth due to two affected elder siblings.
Moreover, one child was in the 1.500-2.000 g birth weight category, two children were in the 2.000 - 2.500g group, and nine children weighed more than 2500g when born.

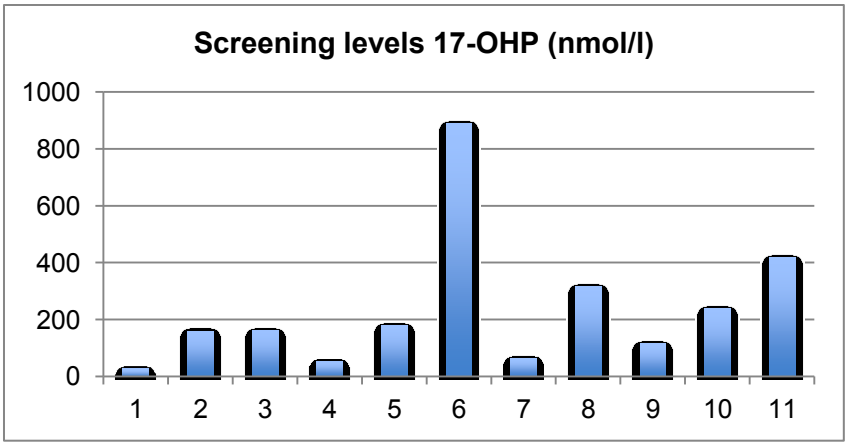

Figure 18: Screening levels 17-OHP in Styrian patients
Screening 17-OHP levels in eleven patients out of twelve for whom newborn screening was available. In one patient 17-OHP measurement was not available.

13.7 Laboratory values

Lowest 17α-hydroxyprogesterone level reached in one patient on average was 0,54 ng/ml, highest was 195,75 ng/ml in one patient on average. According to the standard values of the Universitätsklinikum Graz, range of 17α-hydroxyprogesterone should be between 0,2 and 0,9 ng/ml. In three patients, average 17-OHP levels were between 100 and 200 ng/ml, in three lower than 20 ng/ml, in nine lower than 5 mg/dl, and two patients were in the normal range from 0,2 to 0,9 ng/ml on average.

For the eighteen patients suffering from CAH, a total of 296 laboratory values of 17α-hdroxyprogesterone were analysed. 119 values were within the normal range, which is 40.2% of all values.

13.8 Bone maturity/age

Bone maturity was measured by means of x-ray of the left hand and analysed with the Greulich & Pyle method. (62)

13.8.1 Boys

Four out of six boys showed abnormal bone maturity. Only one boy had normal bone age, and in one case the child is still very young, hence there has not been a radiologic evaluation yet.

The average deviation of bone age from chronologic age ranged between +3,25 years and +6,36 years on average in the individual patients. Highest number of deviation was +8 years in one boy, whereas the chronologic age was five years and the bone age was thirteen years. This special case was a boy diagnosed late due too early onset of puberty and rapid growth spurt. Only one boy had both the same chronologic and bone age respectively no difference was detected.

13.8.2 Girls

Six out of eleven girls with available hand x-rays were found to have advanced bone age. One child was too young and bone age was not available yet.

The deviation of bone age from chronologic age in girls ranged from +0,2 years in the least affected to +3,7 years in the most affected patient on average, reflecting advanced bone maturity due to androgen excess. Highest deviation of bone maturity from chronologic age was +4.2 years in one female patient.

Moreover, five girls showed diminished bone maturity. Average levels ranged from -0,14 years to -2,44 years of differentiation in age.

However, six out of eleven female patients and four out of five male patients were affected.

13.9 Height and weight

13.9.1 Birth weight and length

Birth weight was under the 3rd percentile in five infants, under the 10th percentile in one infant, between 25th – 50th percentile in four infants, between 50th – 75th percentile in four infants, and above the 90th percentile in one infant. In one case, birth weight was not available due to late diagnosis and lacking data.

Birth length was under the 3rd percentile in two infants, between 25th – 50th percentile in two infants, between 50th – 75th percentile in five infants, above the 90th percentile in five infants, and above the 97th percentile in one infant. In two cases, data were lacking.

Children were taller than they were heavy. Weight levels were not elevated above the 75th percentile but in one case, height levels were elevated above the 90th percentile in six infants in total.

To analyse and compare weight and height throughout childhood, data in three-year time lags were collected.

13.9.2 Boys

Six boys were in our cohort, from whom four have the salt-wasting form and one the simple-virilising form of 21-hydroxylase deficiency, whereas one boy suffers from 11β-hydroxylase deficiency.

Not-screened boys

Three boys were born before screening was introduced, and three boys were born afterwards.

From the not screened boys, one has the SV and two the SW form. The simple-virilising boy was diagnosed due to increased growth spurt, one salt-waster was diagnosed at birth due to an affected older half-sister, and the other salt-waster was diagnosed due to pubertas praecox.

The SV boy was diagnosed at the age of six. Height SDS in the measured values was between -0,5 and +4,14, weight SDS was between +1,13 and +2,82. There was an early growth spurt and an abrupt growth stagnation detectable in the percentiles, height percentile was between 30,68 and 100, BMI percentile between 86,19 and 98,05.

SDS height	6 years	9 years	12 years	15 years
	+4,14	+4,19	+2,51	-0,5
SDS weight	6 years	9 years	12 years	15 years
	+2,82	+2,58	+2,46	+1,13

The first SW boy was diagnosed at the age of seven, not having shown perilous salt-wasting effects so far. Height SDS in measured values ranged from +2,43 to +3,12, and weight SDS ranged from +1,58 to +1,91. Height percentile was from 99.25 to 99,91, BMI percentile from 70,93 to 82. Height and weight respectively BMI was highly elevated compared to coevals.

SDS height	7 years	9 years	11 years
	+3,12	+2,87	+2,43
SDS weight	7 years	9 years	11 years
	+1,91	+1,58	+1,61

The second SW boy was known to suffer from SW-CAH from birth on. Height SDS was between -0,77 and +2,43, weight SDS was between -1,34 and +1,73. Height percentile was between 21,95 and 99,25, BMI percentile between 23,31 and 83,73. Values in this boy were not as exaggerated as in the two other boys not screened.

SDS height	3 years	6 years	9 years	12 years
	-0,77	+1,47	+2,43	+0,62
SDS weight	3 years	6 years	9 years	12 years
	-1,34	+0,95	+1,73	+0,58

Screened boys

Three boys were born after 2001 and therefore included in newborn screening for CAH. Two boys suffer from classic 21-hydroxylase deficiency with salt-wasting, and one boy suffers from 11β-hydroxylase deficiency.

However the boy with 11β-hydroxylase deficiency was not found in screening because it is not sensitive to this form of CAH. The patient was diagnosed at the age of two due to pubertas praecox. The patient showed height SDS between +1,72 and +5,64, and weight SDS between +1,75 and +3,22. Height percentile reached from 95,68 to 100, and BMI percentile from 44,6 to 93,99. The measured values show a very tall and heavy child compared to coevals, although growth and weight spurt were not as distinctive any more in the last measurement available.

SDS height	3 years	6 years	9 years
	+5,64	+3,06	+1,72
SDS weight	3 years	6 years	9 years
	+3,22	+2,41	+1,75

The two boys with SW-CAH show height SDS from -1,32 to -0,05 respectively +0,25, and weight SDS from -1,61 to 0,00 respectively -1,57. However values are not as extreme as in the 11β-hydroxylase affected boy or in the boys not screened, possibly showing the result of early diagnosis and therapy in 21-hydroxylase deficiency.

SDS height	1 year	3 years
	-1,32	-0,05
	0,25	x
SDS weight	1 year	3 years
	-1,61	0
	-1,57	x

Summing up, two boys were significantly taller than boys of the same age, with height and weight levels significantly elevated above the 97th percentile on average. These patients were the one with simple-virilising CAH and the one with 11β-hydroxylase deficiency.
One boy with SW-CAH was between 90th and 97th percentile concerning weight, and above 97th percentile concerning height on average.
One boy with SW-CAH was under the 10th percentile in early childhood on average, respectively under the 50th percentile concerning height and under the 75th percentile concerning weight when older.
One boy with SW-CAH was at the 50th percentile both concerning height and weight on average.
One boy with SW-CAH was under the 50th respectively 25th percentile concerning height and weight on average.

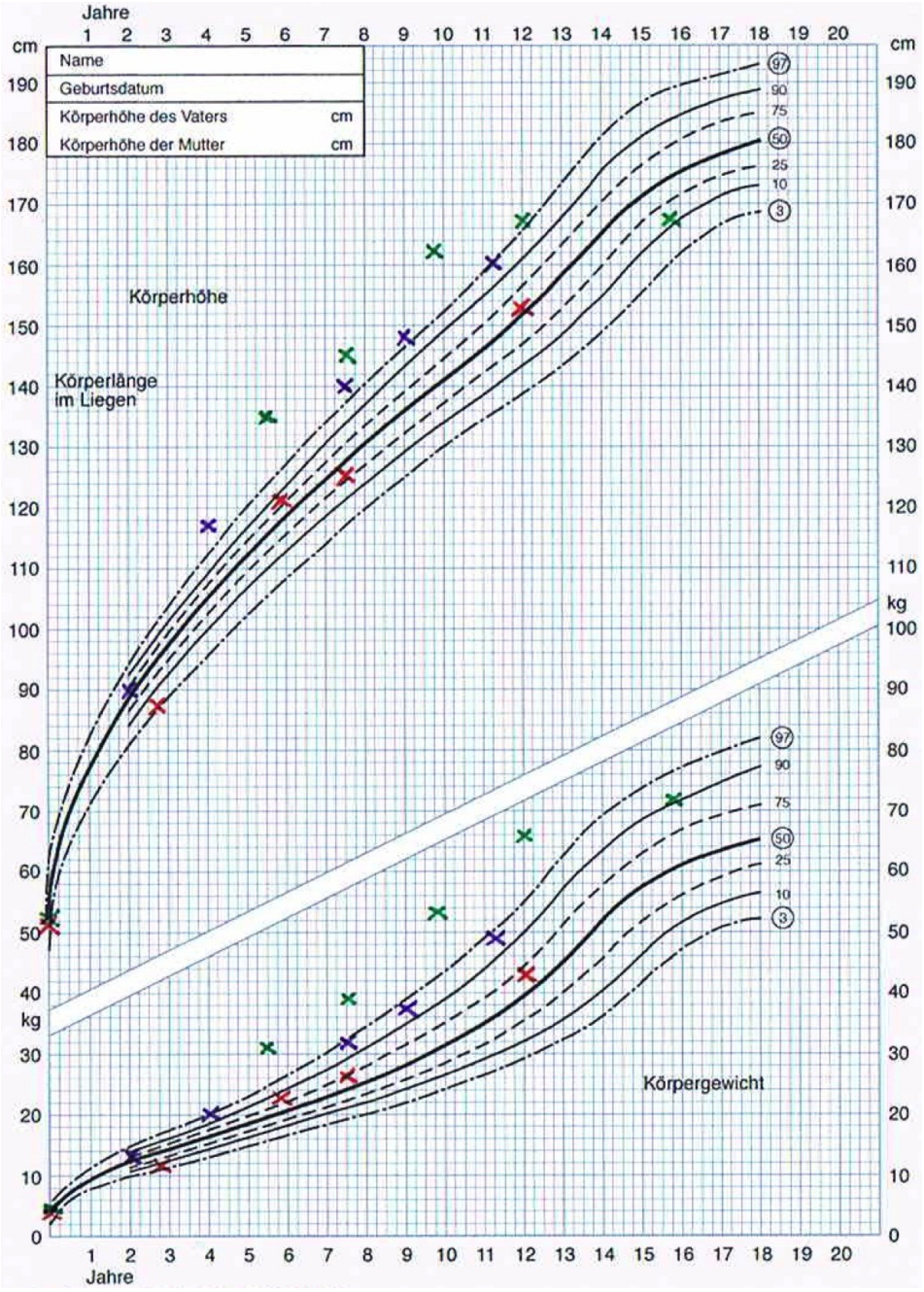

Figure 19: Weight and height percentiles in boys not screened

Green = SV boy

Purple = SW boy 1

Red = SW boy 2

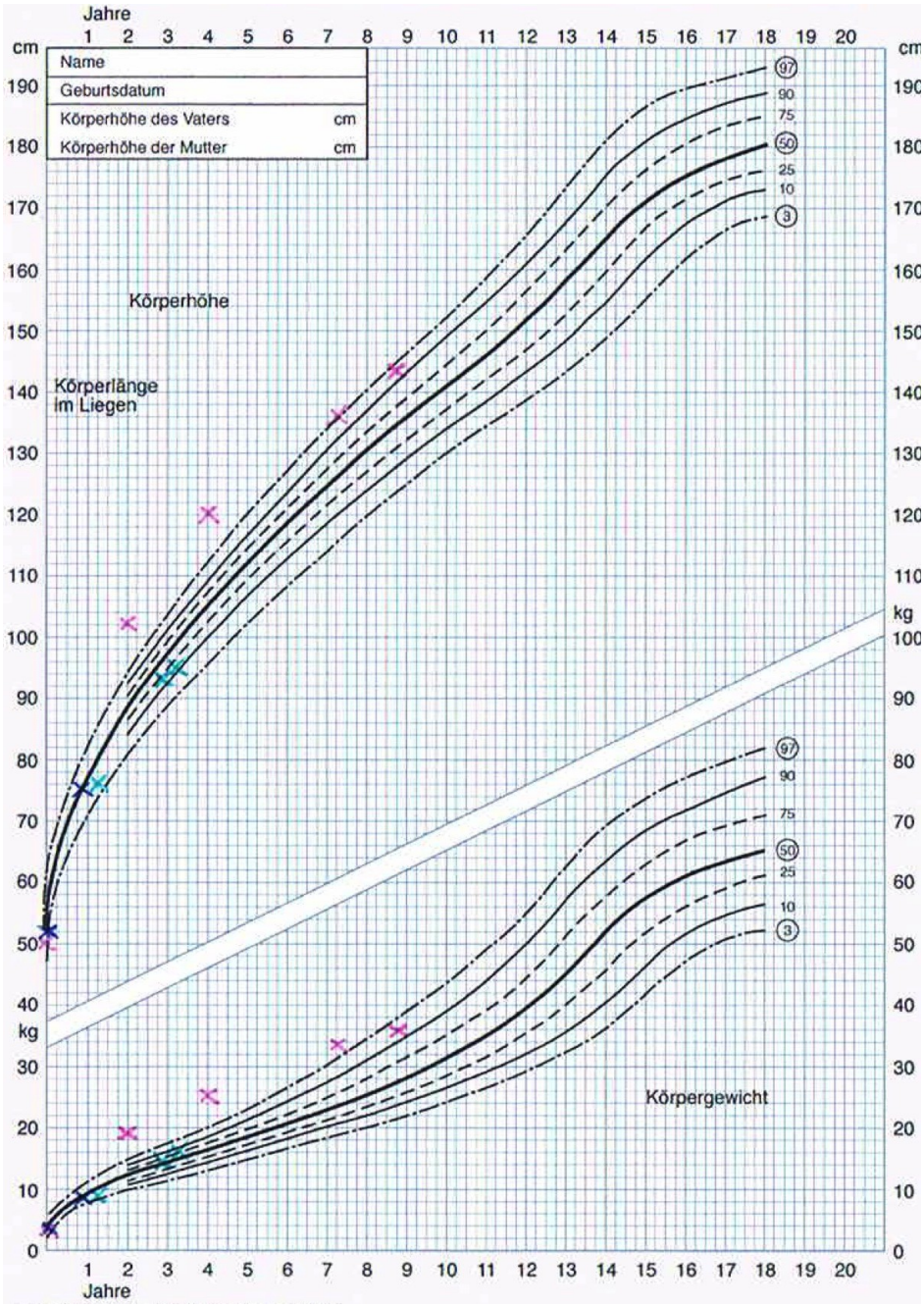

Figure 20: Weight and height percentiles in boys screened

Pink = 11β deficiency

Turquois = SW boy 1

Blue = SW boy 2

13.9.3 Girls

There were twelve girls in our cohort. Eight suffer from salt-wasting 21-hydroxylase deficiency, three from simple-virilising 21-hydroxylase deficiency, and one from 11β-hydroxylase deficiency.

Not-screened girls

Three girls were born before screening was introduced. One of them has the SV from, and two the SW form of 21-hydroxylase deficiency.

The SV girl was diagnosed early due to an affected sister. Height SDS in the measured values was between +0,28 and +2,08. Weight SDS was between +0,48 and +1,87. Height percentile ranged from 61,14 to 98,12, and BMI percentile from 48,04 to 95,15.

SDS height	1 year	3 years	6 years	9 years	12 years	15 years
	+0,28	+1,55	+1,09	+2,08	+1,97	+0,78
SDS weight	1 year	3 years	6 years	9 years	12 years	15 years
	+1,42	+1,58	+0,48	+1,87	+1,44	+1,78

The SW patients were diagnosed due to severe virilisation. Height SDS in the measured values reached from -0,32 to -0,08 in one girl, and from -0,57 to +2,45 in the other. Weight SDS was from -0,35 to +1,06 respectively from +0,08 to +2,41. Height percentile ranged between 38,14 and 58,11 in one girl, and between 28,41 and 99,28 in the other; BMI percentile from 47,2 to 94,92 respectively from 70,02 to 99,05.

SDS height	1 year	3 years	6 years	9 years	12 years
	-0,3	0,2	-0,08	-0,32	-0,2
	-0,57	2,14	0,79	2,45	1,38
SDS weight	1 year	3 years	6 years	9 years	12 years
	1,06	0,56	0,57	0,2	-0,35
	0,08	1,56	0,98	1,73	2,41

The SV girl showed values above the 90th percentile concerning weight, and values between 50th and 97th percentile concerning height. In recent measurements, there was a strong weight gain detectable, whereas growth did not augment at the same extent. Therefore BMI levels have increased drastically in this girl.

The SW girls were different in their height and weight development. One showed weight levels mostly between 25th and 50th percentile, and height levels at or under the 3rd percentile. The other girl showed weight levels above the 50th percentile in early childhood and levels widely above the 97th percentile later in childhood. In this girl, there was also a distinctive weight gain detectable in the last measurements, whereas growth was developing non-parallel and height was only augmenting at a little extent.

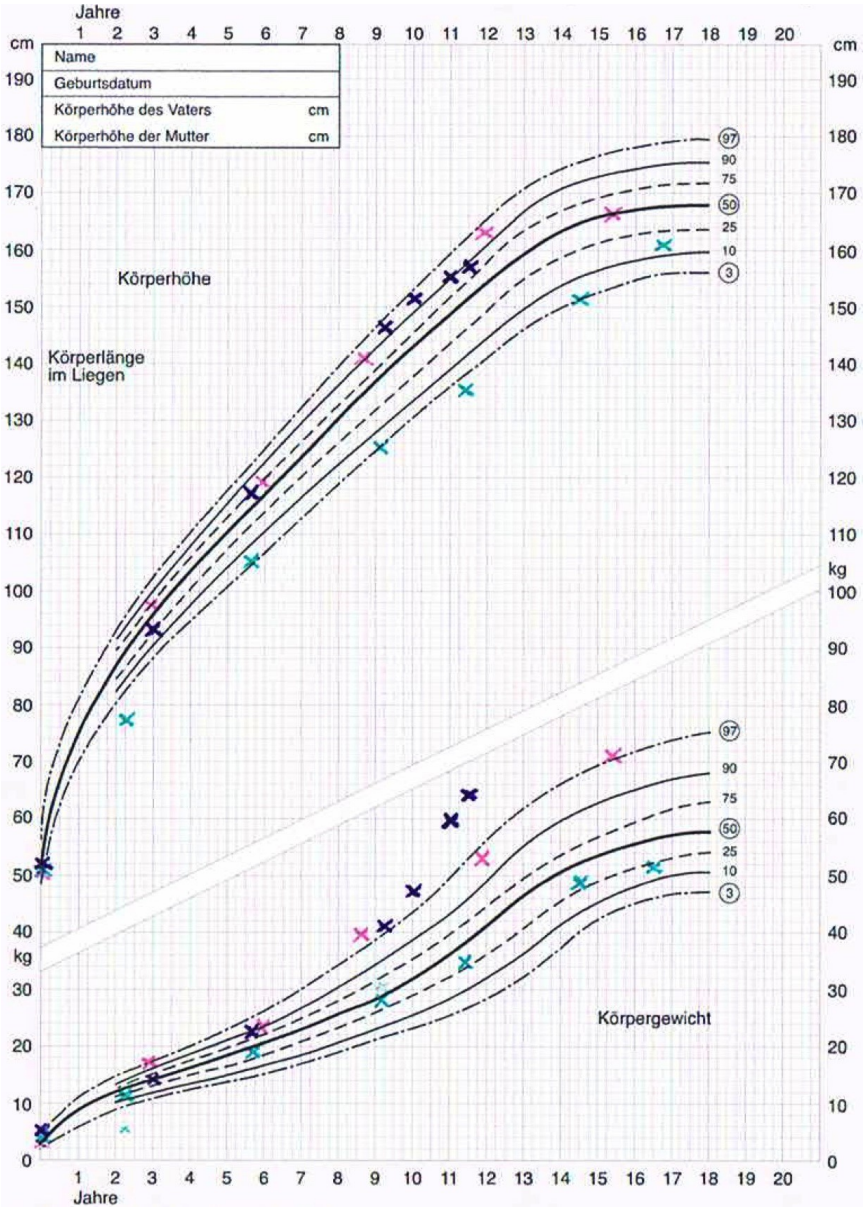

Figure 21: Weight and height percentiles in girls not screened

Pink = SV girl, Turquois = SW girl 1, Purple = SW girl 2

Screened girls

Nine girls were included in newborn screening. Six showed SW-CAH, two SV-CAH and one 11β-hydroxylase CAH. However, only six out of nine girls were diagnosed via screening. One girl with the SV form showed normal 17-OHP levels and therefore was diagnosed at the age of three due to pubertas praecox. One girl showed normal 17-OHP but had already been diagnosed due to affected elder siblings. One girl was diagnosed at the age of several months due to genital ambiguity whereas screening 17-OHP had been normal.

SW 21-hydroxylase deficiency

Five girls were found to have SW-CAH in newborn screening. Screened SW-affected girls were the largest group of patients in our cohort. We collected follow-up data on height and weight every three years.

Height SDS at the age of one year was from -2,22 to -0,37, from -1,67 to -0,17 at three years from +0,09 to +1,21 at six years, and from -0,23 to +1,07 at nine years.

Weight SDS at the age of one year was from -2,75 to +0,16, from -0,93 to +0,74 at three years, from +0.39 to +2,1 at six years, and from +1,19 to + 1,85 at nine years.

SDS height	1 year	3 years	6 years	9 years
	-2,22	-0,24	1,05	1,07
	-2,06	-1,67	0,09	-0,23
	-1,72	-0,17	1,21	x
	-0,81	0,58	0,84	x
	-0,37	-0,34	x	x
	-0,4	x	x	x
SDS weight	1 year	3 years	6 years	9 years
	-2,75	0,74	2,1	1,85
	-2,17	-0,54	1	1,19
	-2,45	-0,84	0,43	x
	-1,36	0,35	0,39	x
	-1,2	-0,93	x	x
	0,16	x	x	x

SV 21-hydroxylase deficiency

The SV girls showed different results concerning weight and height outcome. In the older girl with early diagnosis at birth, height SDS was between +0,78 and +1,84, and weight SDS was between -0,83 and +1,49 in the measured values. Height percentile was between 73,87 and 96,72, and BMI percentile was between 2,83 and 94,49.

In the younger girl with the late diagnosis at three years of age due to pubertas praecox, height SDS ranged from +1,82 to +3,3 and weight SDS from +0,73 to +1,6. Height percentile was between 96,55 and 99,95, and BMI percentile between 2,11 and 84,3. Due to late presentation in the younger and lacking data, and due to difference in age, there are not more values comparable between the two SV girls screened.

SDS height	1 year	3 years	6 years	9 years	12 years
	0,78	1,61	1,84	0,81	0,64
	x	3,3	1,82	x	x
SDS weight	1 year	3 years	5 years	9 years	12 years
	-0,83	0,3	0,88	1,26	1,49
	x	0,73	1,6	x	x

In both girls, measurements in the follow-up examinations at the Kinderklinik of the Medical University of Graz show that height SDS is diminishing and BMI SDS is increasing as the patients grow older, probably predicting a development towards shortness and weight gain as childhood proceeds.

11β-hydroxylase deficiency

The girl with this rare form of CAH showed height SDS between +1,29 and +1,61, and weight SDS between +0,38 and +1,18. Height percentile ranged from 90,14 to 94,64, and BMI percentile from 20,81 to 79,97 in the measured values.

SDS height	1 year	3 years	6 years
	1,44	1,61	1,29
SDS weight	1 year	3 years	6 years
	0,38	0,62	1,18

The measured values show a tall child compared to coevals.

In the following figures, respectively three girls` weight and height percentiles are charted to clearly represent the values.

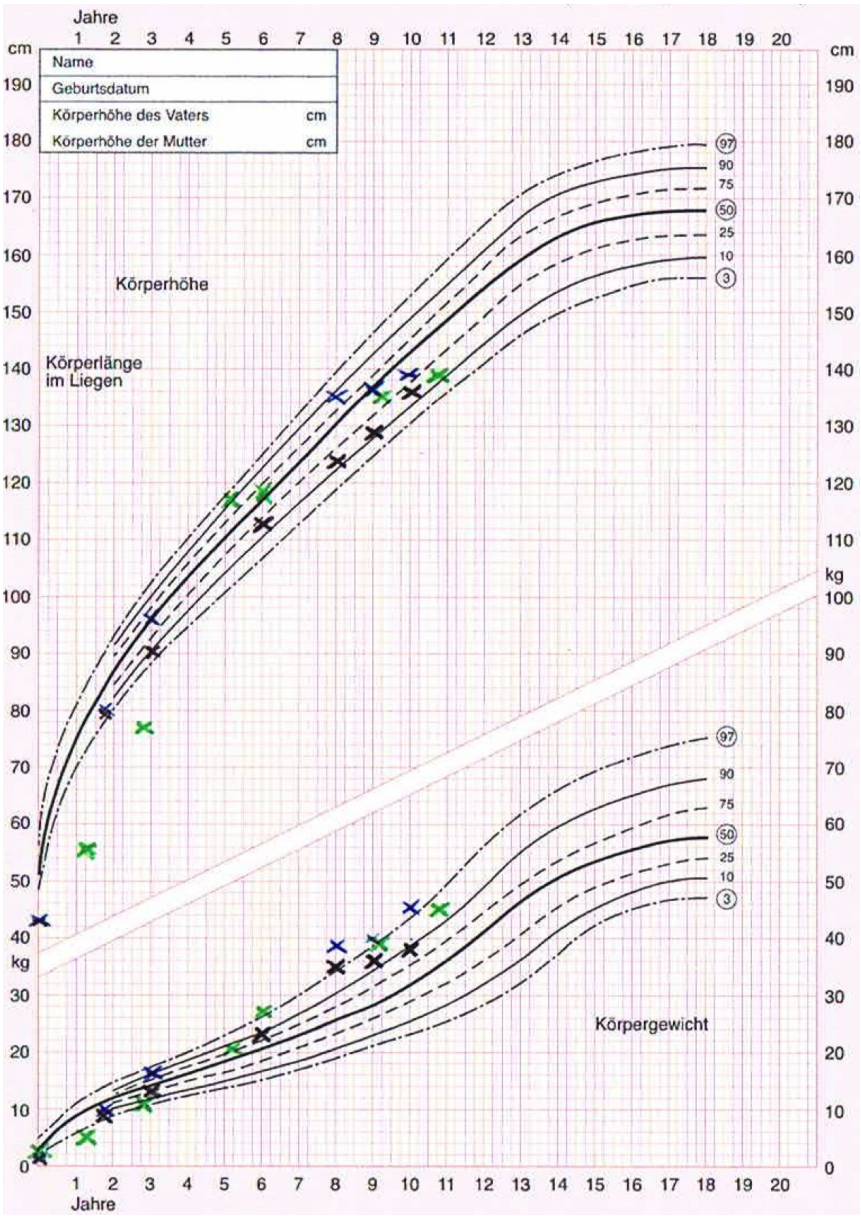

Figure 22: Weight and height percentiles in girls screened 1

Light green = SV girl 1

Blue = SW girl 1

Brown = SW girl 2

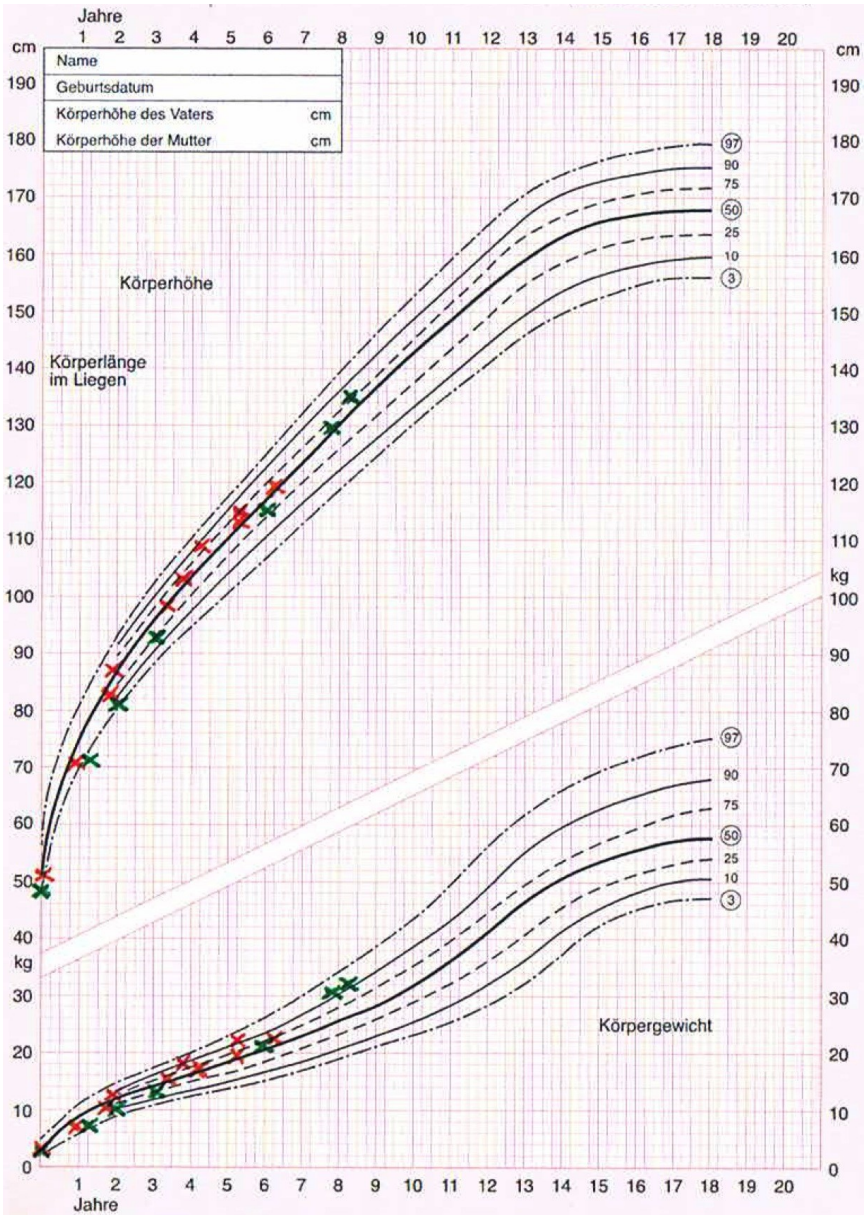

Figure 23: Weight and height percentiles in girls screened 2

Green = SW girl 3

Orange = SW girl 4

Red = 11β girl

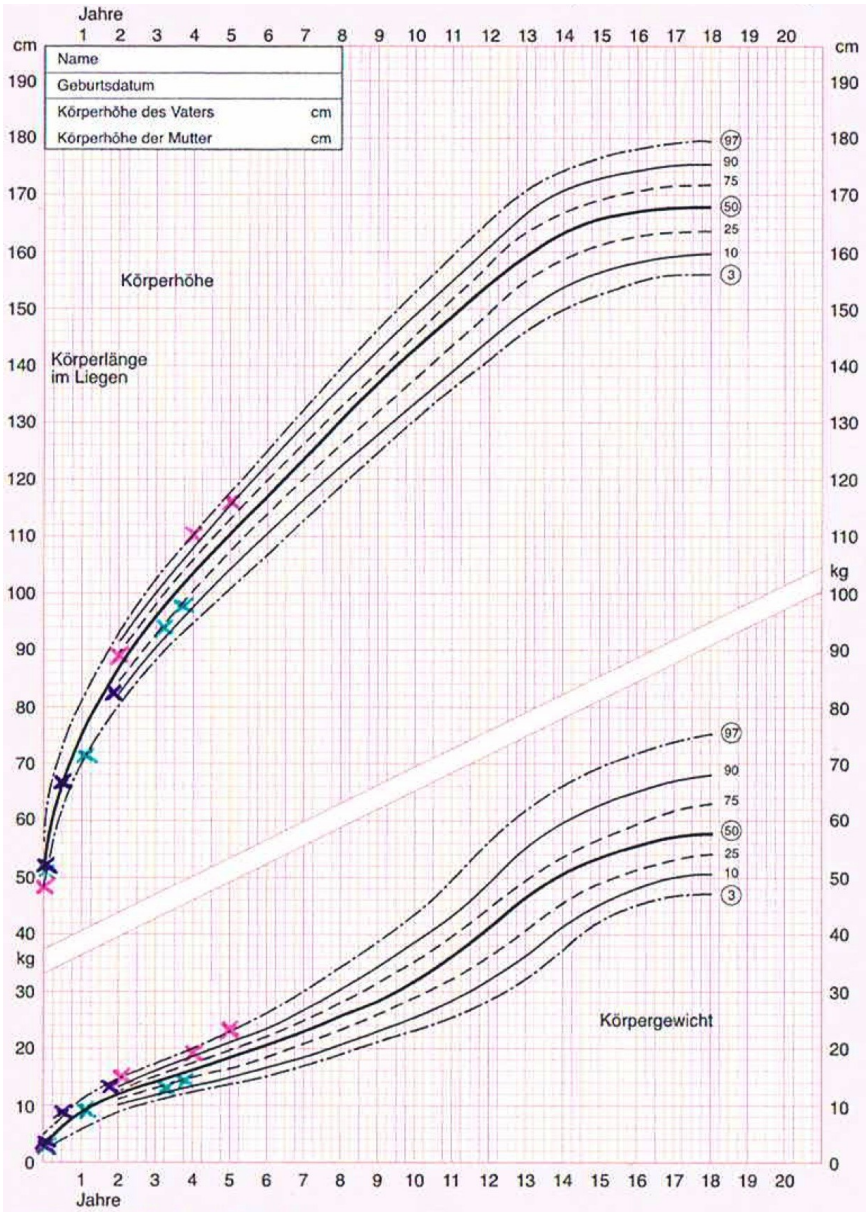

Figure 24: Weight and height percentiles in girls screened 3

Pink = SV girl 2

Turquois = SW girl 5

Purple = SW 6

13.10 Medical treatment

All of our patients, including sixteen patients with 21-hydroxylase deficiency and two with 11β hydroxylase deficiency, were treated with Hydrocortone® to substitute glucocorticoids.
Twelve were treated with Astonin H® in addition to replace mineralocorticoids.
Thirteen patients suffering from congenital adrenal hyperplasia were treated from birth on respectively in the first year of life.
Three male patients were treated from the 2nd, 5th and 7th year of life. However, one of them suffered from the SW form and one from the SV form of 21-hydroxylase deficiency, and one had 11β-hydroxylase deficiency.
Two female patient were treated from the 1st respectively 3rd year of life, having shown normal screening 17-OHP levels at birth, only later on emerging with clitoral hypertrophy and pubic hair. It is distinctive that both of these girls suffered from the SV form of 21-hydroxylase deficiency.
All children have received adequate and quick therapy according to latest guidelines on CAH at the Universitätsklinik für Kinder- und Jugendheilkunde Graz as soon as diagnosis was confirmed. (33)

14 Discussion

Congenital adrenal hyperplasia is a frequent autosomal recessive disorder and the most frequent disorder of steroidogenesis. (1)
Therefore it is useful to learn more about the condition as data in Austria respectively Styria have not been collected and analysed in this form before. In this study, we compared congenital adrenal hyperplasia, its diagnosis, therapy and course of disease to other worldwide studies.

If infants or children are affected, CAH may result in life-threatening consequences or at least impaired quality of life due to signs and symptoms. There is a broad spectrum of manifestations that ought to be alarming for the consulting physician. Thus awareness of the condition and knowledge of the handling and therapy is of utmost importance.

The prevalence of congenital adrenal hyperplasia worldwide differs due to ethnic background. In Caucasians, frequency of 21-hydroxylase deficiency, which is the most common form, is estimated to be 1:14,199. (13) Frequency of 11β-hydroxylase deficiency is 1:160,000. (10)
As we could show in our investigation of CAH in the Austrian/Styrian population, prevalence of congenital adrenal hyperplasia is comparable respectively similar to worldwide results. (13,40,50)
Prevalence of CAH was 1:19.833 in group A (children not screened), compared to 1:10.322 (children screened) in group B.
This result claims that newborn screening for CAH seems to increase the rate of detection of CAH respectively the prevalence of CAH in the population.

As CAH is an autosomal recessive disorder, frequency of manifest CAH in descendants is expected to be one in four respectively 25%, and frequency of carrier status is estimated to be 50%. (8)
However, we found that this relation is not the case in many siblings in our cohort. There are more children affected than one in four on average.
Among our collective, there is also a family where four out of four children are affected with classic CAH, respectively 100%. Moreover, two more children with 21-hydroxylase deficiency in our cohort are siblings, and the two children with 11β-hydroxylase deficiency are also siblings. Affected older or younger siblings exist in at least three patients.

The genetic alterations causing congenital adrenal hyperplasia have been identified to a great extent. (1) As little as 10 mutations cause more than 90% of CAH cases. (27)
Most CAH patients are described as compound heterozygotes inheriting respectively one defect allele of each parent. The enzyme deficiency is as severe as the mildest mutation and rest activity depends on the underlying alteration. (10)
In our study, we could verify that the most patients carry two different mutations, whereas deletions are not as frequent as mutations. Results of Austrian/Styrian genetic analysis are therefore comparable to findings in literature.

Time of diagnosis was compared among children who were included in newborn screening available since 2001, and children who were not screened hence diagnosed by clinical findings or through affected elder siblings.

Generally, it emerges that the introduction of screening leads to earlier diagnosis and treatment, and that it is especially useful for male newborn children that may not show virilisation at birth. In times without screening, they were often underdiagnosed.
In our male patients who were not screened, we found two cases of late diagnosis and accordant medical findings, such as elevated bone age, precocious puberty and short stature. In our male patients screened and hence diagnosed and treated early, height outcome seems to rise and bone age seems unaffected. It is important to identify children with CAH as soon as possible to prevent severe salt-wasting ad obtain appropriate height outcome.

Girls are typically diagnosed due to ambiguous genitalia, being the most distinctive symptom of androgen excess due to underlying congenital adrenal hyperplasia, and being an immediate diagnostic feature at birth. Therefore girls were already diagnosed early before the introduction of screening mostly to genital alterations. However one girl not screened was unaffected at birth, but diagnosed due to an older affected sister. Benefit of screening is greatest in girls with a low grade of virilisation such as Prader I that could be overseen or ignored easily.

However it must be said, that screening cannot identify 100% of affected infants. There are still underdiagnosed children that do not show the typical genital symptoms and laboratory values at birth. Moreover, one must take into account that screening is only sensitive to 21-hydroxylase deficiency. The second most common form, 11β-hydroxylase deficiency, may not be found in screening. Moreover, the non-classic forms often show normal clinical findings and 17-OHP levels at birth. The very rare forms cannot be detected as well. Screening is to be handled carefully in preterm infants, because they normally show elevated hormone levels, and weight respectively gestational age adjustment of screening levels is indispensable, (49,51–53)
These above mentioned conditions mark the most important weak points of newborn screening according to our investigations and findings. Three of our patients with CAH were not diagnosed despite screening; two girls who were screened and not found to have SV 21-hydroxylase deficiency, and one boy screened and not found to suffer from 11β-hydroxylase deficiency. Of course there are many benefits of screening, but we may not forget that screening is not inerrant and we cannot rely on it in any case.

Concerning the sex ratio, boys and girls should be affected at the same extent. (3)
However, we found that gender ratio is shifted towards females in the screened group, where it is balanced in the not-screened group. In the screened group, more girls were diagnosed with CAH than boys. The ratio is 2:1, whereas it is 1:1 in the not-screened group.

Concerning the ratio of SV and SW form, it is claimed that the ratio of affected patients is 3:1 or respectively that 75% have the SW and 25% the SV form. (5)
We also found this ratio in our cohort, with twelve SW patients and four SV patients out of sixteen patients with CAH due to 21-hydroxylase deficiency. In the screened group SW:SV ratio was 5:1, and in the not-screened group it was 2:1. In total (both groups), it was exactly 3:1, with twelve identified “salt-wasters” and four “simple-virilisers”.

Height and weight outcome were investigated in four groups: boys not screened, boys screened, girls not screened and girls screened.
It was remarkable that the boy who was not screened and had the SV form respectively the boy who was not found to have 11β-hydroxylase deficiency in screening grew fast and were very tall compared to coevals. However these growth spurts ended as childhood proceeded and percentiles predict short stature compared to coevals. This correlates with the finding that these children grow fast in early childhood and stop growing in advanced childhood, not reaching their parental target height. (18)

The boys who were found to suffer from CAH in screening are still very young, but have been diagnosed early and thus treated since birth. Their weight and height percentiles are not as exaggerated as in the older male patients, and the data show an average respectively slightly below average height and weight development.

Two of the three girls who were not screened were diagnosed due to severe virilisation (Prader IV and V). One girl was diagnosed due to an effected elder sibling and consecutive control. However, height and weigh in two of them showed tall children in early childhood, but growth stop and increased BMI in advanced childhood. Height and weight outcome is not optimal despite early diagnosis and treatment, as it has been described in literature. (27) The girls who were screened also tended to show early growth spurt, subsequent growth stop and increasing BMI, probably predicting short stature and overweight in adulthood. One girl with SV 21-hydroxylase deficiency was not identified in screening and showed excessive growth spurt, whereas weight appeared within normal range.

Generally, the extent of virilisation in girls reached from unaffected to severe virilisation with a penile urethra, according to Prader V. However, degree of virilisation was various in both groups, the screened and the not-screened girls. Moreover, degree of virilisation did not correlate with the SV or SW form, respectively degree of virilisation did not correlate with severity of enzyme deficiency.

Bone age was elevated in boys rather than in girls. Six out of eleven female patients with available x-rays showed advanced bone age, whereas it was four out of five male patients. Moreover, the measured deviations of bone age from chronologic age had a wider span in male patients. The presentation of advanced bone age as a typical symptom of CAH was existent in Styrian patients as expected and described in literature. (6,10,22)

The medical condition of congenital adrenal hyperplasia is explored well nowadays. As screening is available in several countries, the diagnosis is made earlier and therapy started immediately. (40,50)
As a consequence of screening offered, the outcome is better respectively the effects of the disease are restricted in the affected children. Therefore, quality of life is consequently improving for the patients.

However, there are still some problems remaining that pose a challenge to modern endocrinology medicine. Among these count diminished height and impaired fertility both in men and women, as well as so-called testicular adrenal rest tumours in males. (27)

Therapy methods have been established both in glucocorticoid and mineralocorticoid replacement, and there are guidelines that are updated by diverse endocrine societies on a regular basis in order to guarantee optimal treatment in affected children. (24,32,33)
The patients in Graz were treated according to these guidelines, and were given both Hydrocortone® and Astonin H® to achieve adequate final height and suppress androgens. However, weight issues and low height are rather common among CAH patients, even if therapeutic intervention was sufficient and compliance was guaranteed. This may result from both under- and overtreatment. (55) Therefore, the substitution of hormones has to be supervised by a specialist.

Besides the therapeutic measures and medical considerations, the medical consultant should be aware that the condition of congenital adrenal hyperplasia can be a severe impact on life both in patients and their families, and the issue is to be handled carefully and sensitive.

A good outcome depends on decent therapy, which requires constant compliance and follow-up at a specialized centre such as the Universitätsklinik für Kinder- und Jugendheilkunde Graz.

15 References

1. Michael P. Wajnrajch, Maria I. New. Defects of Adrenal Steroidogenesis. In: Leslie J. DeGroot, J. Larry Jameson, editors. Endocrinology. Fifth edition. Philadelphia, PA: Elsevier Saunders; 2005. p. 2393–415.

2. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. The Lancet. 2005 Jun;365(9477):2125–36.

3. Bartmann P, Sitzmann FC. Pädiatrie : 300 Tabellen. Stuttgart: Thieme; 2012.

4. test.classconnection.s3.amazonaws.com.webloc. [19.2.2013]

5. Speiser PW, White PC. Congenital Adrenal Hyperplasia. New England Journal of Medicine. 2003 Aug 21;349(8):776–88.

6. New MI. An update of congenital adrenal hyperplasia. Ann. N. Y. Acad. Sci. 2004 Dec;1038:14–43.

7. causes.aspx [Internet]. [cited 2013 Jan 31]. Available from: https://www.nichd.nih.gov/health/topics/cah/conditioninfo/pages/causes.aspx

8. Murken J. Taschenlehrbuch Humangenetik : 96 Tabellen. Stuttgart [u.a.]: Thieme; 2006.

9. Silbernagl S, Lang F. Taschenatlas der Pathophysiologie. Stuttgart; New York: Thieme; 2005.

10. Allolio B. Praktische Endokrinologie. München: Elsevier, Urban & Fischer; 2010.

11. Pang S, Clark A, Neto EC, Giugliani R, Dean H, Winter J, et al. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: Newborn screening and its relationship to the diagnosis and treatment of the disorder. Screening. 1993 Apr;2(2-3):105–39.

12. Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI. High frequency of nonclassical steroid 21-hydroxylase deficiency. Am. J. Hum. Genet. 1985 Jul;37(4):650–67.

13. Pang SY, Wallace MA, Hofman L, Thuline HC, Dorche C, Lyon IC, et al. Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics. 1988 Jun;81(6):866–74.

14. Böcker W, Aguzzi A. Pathologie. München [etc.]: Urban & Fischer; 2008.

15. Krone N. Predicting Phenotype in Steroid 21-Hydroxylase Deficiency? Comprehensive Genotyping in 155 Unrelated, Well Defined Patients from Southern Germany. Journal of Clinical Endocrinology & Metabolism. 2000 Mar 1;85(3):1059–65.

16. http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/adrenal/cah.html. [19.2.2013]

17. New MI. Nonclassical 21-Hydroxylase Deficiency. Journal of Clinical Endocrinology & Metabolism. 2006 Sep 5;91(11):4205–14.

18. New MI, Wilson RC. Inaugural Article: Steroid disorders in children: Congenital adrenal hyperplasia and apparent mineralocorticoid excess. Proceedings of the National Academy of Sciences. 1999 Oct 26;96(22):12790–7.

19. Donaldson MD, Thomas PH, Love JG, Murray GD, McNinch AW, Savage DC. Presentation, acute illness, and learning difficulties in salt wasting 21-hydroxylase deficiency. Archives of Disease in Childhood. 1994 Mar 1;70(3):214–8.

20. Kovacs J. Lessons From 30 Years of Clinical Diagnosis and Treatment of Congenital Adrenal Hyperplasia in Five Middle European Countries. Journal of Clinical Endocrinology & Metabolism. 2001 Jul 1;86(7):2958–64.

21. 21-Hydroxylase-Deficient Nonclassic Adrenal Hyperplasia: The Great Pretender. Seminars in Reproductive Medicine. 2003;21(3):295–300.

22. Lehnert H, Begum N, Deutsche Gesellschaft für Endokrinologie. Rationelle Diagnostik und Therapie in Endokrinologie, Diabetologie und Stoffwechsel 154 Tabellen. Stuttgart; New York, NY: Thieme; 2010.

23. Gynäkologie und Geburtshilfe. Berlin: Springer; 2006.

24. Joint LWPES/ESPE CAH Working Group. Consensus Statement on 21-Hydroxylase Deficiency from The Lawson Wilkins Pediatric Endocrine Society and The European Society for Paediatric Endocrinology. Journal of Clinical Endocrinology & Metabolism. 2002 Sep 1;87(9):4048–53.

25. Sun S, Zhang M, Yang J, Zhang H, Liu J, Hong J, et al. [Clinical and genetic analysis of 11β-hydroxylase deficiency]. Zhonghua Yi Xue Za Zhi. 2011 Nov 15;91(42):2999–3002.

26. Hochberg Z, Schechter J, Benderly A, Leiberman E, Rosler A. Growth and pubertal development in patients with congenital adrenal hyperplasia due to 11-beta-hydroxylase deficiency. Am. J. Dis. Child. 1985 Aug;139(8):771–6.

27. Oxford handbook of endocrinology and diabetes. 2nd ed. Oxford ; New York: Oxford University Press; 2009.

28. Saenger P. Prenatal diagnosis of congenital lipoid adrenal hyperplasia. Journal of Clinical Endocrinology & Metabolism. 1995 Jan 1;80(1):200–5.

29. Bose HS. Mutations in the Steroidogenic Acute Regulatory Protein (StAR) in Six Patients with Congenital Lipoid Adrenal Hyperplasia. Journal of Clinical Endocrinology & Metabolism. 2000 Oct 1;85(10):3636–9.

30. Miller W. Congenital lipoid adrenal hyperplasia: the human gene knockout for the steroidogenic acute regulatory protein. Journal of Molecular Endocrinology. 1997 Dec 1;19(3):227–40.

31. Balen AH. Reproductive endocrinology for the MRCOG and beyond. London: RCOG Press; 2007.

32. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, et al. A Summary of the Endocrine Society Clinical Practice Guidelines on Congenital Adrenal Hyperplasia due to Steroid 21-Hydroxylase Deficiency. International Journal of Pediatric Endocrinology. 2010;2010:1–5.

33. Klaus Kapelari. Adrenogenitales Syndrom (AGS) Leitlinie März 2006.

34. New MI. EXTENSIVE PERSONAL EXPERIENCE: Prenatal Diagnosis for Congenital Adrenal Hyperplasia in 532 Pregnancies. Journal of Clinical Endocrinology & Metabolism. 2001 Dec 1;86(12):5651–7.

35. Korth-Schutz S, Virdis R, Saenger P, Chow DM, Levine LS, New MI. Serum Androgens as a Continuing Index of Adequacy of Treatment of Congenital Adrenal Hyperplasia. Journal of Clinical Endocrinology & Metabolism. 1978 Mar 1;46(3):452–8.

36. Claahsen-van der Grinten HL, Stikkelbroeck NMML, Otten BJ, Hermus ARMM. Congenital adrenal hyperplasia--pharmacologic interventions from the prenatal phase to adulthood. Pharmacol. Ther. 2011 Oct;132(1):1–14.

37. Seckl JR. How Safe Is Long-term Prenatal Glucocorticoid Treatment? JAMA: The Journal of the American Medical Association. 1997 Apr 2;277(13):1077.

38. Bongiovanni AM, Eberlein WR, Goldman AS, New M. Disorders of adrenal steroid biogenesis. Recent Prog. Horm. Res. 1967;23:375–449.

39. Zerah M, Pang SY, New MI. Morning salivary 17-hydroxyprogesterone is a useful screening test for nonclassical 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. 1987 Aug;65(2):227–32.

40. Van der Kamp H. Neonatal screening for congenital adrenal hyperplasia. European Journal of Endocrinology. 2004 Nov 1;151(Suppl_3):U71–U75.

41. Therrell BL. Newborn screening for congenital adrenal hyperplasia. Endocrinol. Metab. Clin. North Am. 2001 Mar;30(1):15–30.

42. New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack MS, et al. Genotyping Steroid 21-Hydroxylase Deficiency: Hormonal Reference Data. Journal of Clinical Endocrinology & Metabolism. 1983 Aug 1;57(2):320–6.

43. Joehrer K, Geley S, Strasser-Wozak EMC, Azziz R, Wollmann HA, Schmitt K, et al. CYP11B1 Mutations Causing Non-Classic Adrenal Hyperplasia due to 11 -Hydroxylase Deficiency. Human Molecular Genetics. 1997 Oct 1;6(11):1829–34.

44. Johannsen TH. Delayed Diagnosis of Congenital Adrenal Hyperplasia with Salt Wasting Due to Type II 3 -Hydroxysteroid Dehydrogenase Deficiency. Journal of Clinical Endocrinology & Metabolism. 2005 Jan 18;90(4):2076–80.

45. Kagimoto M, Winter JSD, Kagimoto K, Simpson ER, Waterman MR. Structural Characterization of Normal and Mutant Human Steroid 17 - Hydroxylase Genes: Molecular Basis of One Example of Combined 17 - Hydroxylase/17,20 Lyase Deficiency. Molecular Endocrinology. 1988 Jun 1;2(6):564–70.

46. Hashemipour M, Ghasemi M, Hovsepian S. A case of congenital lipoid adrenal hyperplasia. Int J Prev Med. 2012 Jul;3(7):510–4.

47. New MI. 21-hydroxylase deficiency congenital adrenal hyperplasia. The Journal of Steroid Biochemistry and Molecular Biology. 1994 Jan;48(1):15–22.

48. Kasper DC, Ratschmann R, Metz TF, Mechtler TP, Möslinger D, Konstantopoulou V, et al. The National Austrian Newborn Screening Program – Eight years experience with mass spectrometry. Past, present, and future goals. Wiener klinische Wochenschrift. 2010 Oct 15;122(21-22):607–13.

49. Zabransky S. Screening auf angeborene endokrine und metabole Störungen : Methoden, Anwendung und Auswertung. Wien [u.a.]: Springer; 2001.

50. Kaye CI, And the Committee on Genetics. Newborn Screening Fact Sheets. PEDIATRICS. 2006 Sep 1;118(3):e934–e963.

51. Nordenstrom A, Wedell A, Hagenfeldt L, Marcus C, Larsson A. Neonatal Screening for Congenital Adrenal Hyperplasia: 17-Hydroxyprogesterone Levels and CYP21 Genotypes in Preterm Infants. PEDIATRICS. 2001 Oct 1;108(4):e68–e68.

52. Peter M. Congenital Adrenal Hyperplasia: 11β-Hydroxylase Deficiency. Seminars in Reproductive Medicine. 2002;20(3):249–54.

53. Peter M, Janzen N, Sander S, Korsch E, Riepe FG, Sander J. A Case of 11β-Hydroxylase Deficiency Detected in a Newborn Screening Program by Second-Tier LC-MS/MS. Hormone Research. 2008;69(4):253–6.

54. Bonfig W, Bechtold S, Schmidt H, Knorr D, Schwarz HP. Reduced Final Height Outcome in Congenital Adrenal Hyperplasia under Prednisone Treatment: Deceleration of Growth Velocity during Puberty. Journal of Clinical Endocrinology & Metabolism. 2007 Feb 13;92(5):1635–9.

55. Manoli I, Kanaka-Gantenbein C, Voutetakis A, Maniati-Christidi M, Dacou-Voutetakis C. Early growth, pubertal development, body mass index and final height of patients with congenital adrenal hyperplasia: factors influencing the outcome. Clinical Endocrinology. 2002 Oct 22;57(5):669–76.

56. Eugster EA, DiMeglio LA, Wright JC, Freidenberg GR, Seshadri R, Pescovitz OH. Height outcome in congenital adrenal hyperplasia caused by 21-hydroxylase deficiency: A meta-analysis. The Journal of Pediatrics. 2001 Jan;138(1):26–32.

57. Stikkelbroeck NMML. Growth Inhibition by Glucocorticoid Treatment in Salt Wasting 21-Hydroxylase Deficiency: In Early Infancy and (Pre)Puberty. Journal of Clinical Endocrinology & Metabolism. 2003 Aug 1;88(8):3525–30.

58. Volkl TMK. Obesity Among Children and Adolescents With Classic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency. PEDIATRICS. 2006 Jan 1;117(1):e98–e105.

59. Cameron FJ, Kaymakci B, Byrt EA, Ebeling PR, Warne GL, Wark JD. Bone mineral density and body composition in congenital adrenal hyperplasia. J. Clin. Endocrinol. Metab. 1995 Jul;80(7):2238–43.

60. Arlt W, Willis DS, Wild SH, Krone N, Doherty EJ, Hahner S, et al. Health Status of Adults with Congenital Adrenal Hyperplasia: A Cohort Study of 203 Patients. Journal of Clinical Endocrinology & Metabolism. 2010 Aug 18;95(11):5110–21.

61. Mora S, Saggion F, Russo G, Weber G, Bellini A, Prinster C, et al. Bone density in young patients with congenital adrenal hyperplasia. Bone. 1996 Apr;18(4):337–40.

62. Greulich WW, Pyle SI. Radiographic atlas of sceletal development of the hand and wrist. Standford: Standford University Press; 1974.

63. Martin DD, Wit JM, Hochberg Z, Van Rijn RR, Fricke O, Werther G, et al. The Use of Bone Age in Clinical Practice – Part 2. Hormone Research in Paediatrics. 2011;76(1):10–6.

64. Hochberg Z. Bone biology and skeletal maturity in a clinical setting. Basel; New York: Karger; 2002.

65. Reiser M, Born C, Kuhn F-P, Debus J. Radiologie : 104 Tabellen. Stuttgart: Thieme; 2006.

66. Thiemann H-H, Reisinger W. Röntgenatlas der normalen Hand im Kindesalter. Stuttgart; New York: Thieme; 2006.

67. Volkl TMK, Simm D, Dotsch J, Rascher W, Dorr HG. Altered 24-Hour Blood Pressure Profiles in Children and Adolescents with Classical Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. Journal of Clinical Endocrinology & Metabolism. 2006 Sep 26;91(12):4888–95.

68. Hagenfeldt K, Janson PO, Holmdahl G, Falhammar H, Filipsson H, Frisen L, et al. Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Human Reproduction. 2008 Apr 18;23(7):1607–13.

69. Mulaikal RM, Migeon CJ, Rock JA. Fertility rates in female patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N. Engl. J. Med. 1987 Jan 22;316(4):178–82.

70. Bidet M, Bellanne-Chantelot C, Galand-Portier M-B, Golmard J-L, Tardy V, Morel Y, et al. Fertility in Women with Nonclassical Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. Journal of Clinical Endocrinology & Metabolism. 2010 Jan 15;95(3):1182–90.

71. Hague WM, Adams J, Rodda C, Brook CG, De Bruyn R, Grant DB, et al. The prevalence of polycystic ovaries in patients with congenital adrenal hyperplasia and their close relatives. Clin. Endocrinol. (Oxf). 1990 Oct;33(4):501–10.

72. Reisch N, Flade L, Scherr M, Rottenkolber M, Pedrosa Gil F, Bidlingmaier M, et al. High Prevalence of Reduced Fecundity in Men with Congenital Adrenal Hyperplasia. Journal of Clinical Endocrinology & Metabolism. 2009 Mar 3;94(5):1665–70.

73. Falhammar H, Nystrom HF, Ekstrom U, Granberg S, Wedell A, Thoren M. Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia. European Journal of Endocrinology. 2011 Dec 9;166(3):441–9.

74. Statistik Austria. Available from: http://statcube.at/superwebguest/login.do?guest=guest&db=debevstandjb. [19.2.2013]

75. Statistik Austria. Available from: http://statcube.at/superwebguest/login.do?guest=guest&db=degeborene_ext. [19.2.2013]